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The Host Range of the Wheat Curl Mite, Vector of Wheat Streak-Mosaic[1]

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The wheat curl mite, *Aceria tulipae* (K.) (*Acarina, Eriophyidae*), also called the dry bulb mite and the tulip bulb mite, was first described by Kiefer (1937). The type specimens described by Kiefer were collected from tulip bulbs. Until recently *Aceria tulipae* was considered of little importance. Slykhuis (1953), working on wheat streak-mosaic in Alberta, Canada, found it to be a vector of this disease. Later this discovery was confirmed by Staples & Connin (1953). Since the streak-mosaic is a serious disease of wheat, the mite's role as a vector is being investigated in the Great Plains region.

Little work has been done on the biology of *Aceria tulipae* owing to its microscopic size (210 to 250 by 50 to 65 microns) and to lack of previous knowledge concerning its importance. The host range has not been completely determined, but apparently the Gramineae are among the favored hosts. Direct damage to the host plants on the Great Plains under normal conditions is not severe and is confined to a longitudinal rolling of the leaves. The tip of the youngest leaf as it emerges from the sheath is thus often trapped by the curled portion of the preceding leaf.

Painter & Schesser (1954) list western wheatgrass, *Agropyron smithii* Rydb., as a host of *Aceria tulipae* in Kansas. Slykhuis (1955) reported on some greenhouse studies in Alberta, Canada, with a small number of wild and cultivated grasses. The present author has collected adults, nymphs, and eggs of the mite from western wheatgrass and from seeded and volunteer wheat in the field, and he has observed adults, but not nymphs or eggs, in the field on Canada wild-rye, *Elymus canadensis* L., green foxtail, *Setaria viridis* (L.), and smooth crabgrass, *Digitaria ischaemum* (Schreb.). A number of other grasses infected with wheat streak-mosaic have been discovered in the field (Sill & Connin 1953). McKinney & Fellows (1951) and McKinney & Sando (1951) have reported on additional grasses susceptible to wheat streak-mosaic when manually inoculated under greenhouse conditions.

A study of the host range of this mite in greenhouses at Manhattan, Kan., during the winter of 1953–4 is reported in this paper.

GENERAL PROCEDURE.—As many wild and cultivated grasses as were immediately available were tested as hosts for the mite. The plants were grown in 4- or 6-inch flower pots placed in 6-inch saucers and watered from the bottom. Most of the tests were replicated, and from 2 to 7 vigorous plants were grown in each pot. Leaf sections from wheat plants heavily infested with *Aceria tulipae* were placed in the whorls or over the leaves of the test plants. In a portion of the work the mites were cultured on wheat diseased with the streak-mosaic, and observations were made on the transmission of the disease to the host plants. The development of the mites on the plants was compared with that on the wheat selection 462666 (Marquillo-Oro X Pawnee, C. I. No. 12851), a favorable host for reproduction and susceptible to the streak-mosaic as transmitted by the mite. Temperatures varied between 68° and 90° F. The plants were examined under a 30-power binocular microscope.

WILD GRASSES.—Seed from 24 species of wild grasses was planted at various times, and 10 to 14 days after germination the plants were infested with viruliferous mites, all the plants of each replication on the same day. Sections of leaves were examined under the microscope after 3 to 4 days, and entire plants after 7, 14, and 21 days. Mites were often found moving about on the plant after 3 to 4 days or even after 7 days. However, on half the grasses studied no eggs or very small forms were observed. Their presence after 14 or 21 days was considered evidence that the plant was providing a satisfactory host for reproduction.

The data obtained on wild grasses as hosts for the mite are given in table 1. The mite was able to live and reproduce on 12 of these grasses. Goatgrass, western wheatgrass, an unidentified grama grass, sandbur, smooth crabgrass, Canada wild-rye, and Johnson grass were fairly acceptable hosts when compared with the wheat check. In all these grasses the characteristic rolling and trapping of leaves caused by the mite was present.

The mites did a little feeding on the species not suitable as reproductive hosts, as evidenced by the fact that green foxtail and ticklegrass became diseased. Mites never were observed on either of these grasses more than 4 days after infestation, and no eggs ever were observed on them. It is possible, therefore, that some of the other grasses indicated as unsuitable for increase of the mite could sustain it for short periods.

[1] Accepted for publication May 31, 1955. Contribution No. 635, Department of Entomology, Kansas Agricultural Experiment Station, Manhattan. This work is part of a cooperative project between the Entomology Research Branch and the Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and the Kansas Agricultural Experiment Station.

Table 1.—Reaction of wild grasses to infestation by viruliferous wheat curl mites under greenhouse conditions.

Scientific Name	Grass[a] Common Name	Habit[b]	Growing Season	Number of Replicates	Increase of Mites	Disease Transmitted by Mites
Aegilops cylindrica Host.	Jointed goatgrass	A	May–June	4	Fair-good	Yes
Agropyron elongatum Beauv.	Tall wheatgrass	P	May–Aug.	8	None	No[c]
Agropyron smithii Rydb.	Western wheatgrass	P	May–Aug.	6	Poor-fair	No[c]
Alopecurus pratensis L.	Meadow foxtail	P	June–July	1	None	No
Arrhenatherum elatius L.	Tall oatgrass	P	June–July	1	Poor	No
Bouteloua curtipendula (Michx.)	Sideoats grama	P	July–Sept.	10	None	No
Bouteloua gracilis (H.B.K.)	Blue grama	P	—	10	None	No
Bouteloua sp. (possibly *hirsuta*)	Grama	P	—	4	Good	Yes
Bromus inermis Leyss.	Smooth brome	P	May–Aug.	4	Very poor	No[c]
Bromus tectorum L.	Downy chess	A	May–July	1	None	No
Cenchrus pauciflorus Benth.	Sandbur	A	July–Oct.	3	Good	Yes
Dactylis glomerata L.	Orchard grass	P	May–June	1	None	No
Digitaria ischaemum (Schreb.)	Smooth crabgrass	A	July–Sept.	5	Fair-good	Yes
Digitaria sanguinalis (L.)	Crabgrass	A	June–Sept.	4	Very poor	Yes
Eleusine indica (L.)	Goosegrass	A	June–Sept.	2	None	No[c]
Elymus canadensis L.	Canada wild-rye	P	July–Aug.	2	Fair	No
Eragrostis cilianensis (All.)	Lovegrass	A	June–Sept.	3	Poor	Yes
Euchlaena mexicana Schrad.	Teosinte	A	—	1	Poor	No
Panicum capillare L.	Ticklegrass	A	July–Sept.	4	None	Yes
Panicum virgatum L.	Switchgrass	P	Aug.–Sept.	4	None	No[c]
Phalaris arundinacea L.	Reed canary grass	P	June–Aug.	2	None	No[c]
Setaria viridis (L.)	Green foxtail	A	July–Sept.	3	None	Yes
Sorghum halepense (L.)	Johnson grass	P	July–Oct.	4	Good	No[c]
Sorghastrum nutans (L.)	Indian grass	P	July–Sept.	3	None	No[c]

[a] From "Grasses in Kansas" by F. C. Gates, 1936. Kans. State Bd. Agr. Rpt. LV, No. 220-A.
[b] A, annual; P, perennial.
[c] Host reported to be immune to wheat streak-mosiac virus (Sill and Connin 1953).

Mites removed from the grasses showing increase were able to increase on wheat when returned to that host. Streak-mosaic was transmitted back to wheat from the grass hosts susceptible to the disease.

CULTIVATED GRASSES.—*Wheat.*—The following commercial varieties of wheat were tested as hosts of the wheat curl mite:

Variety	*C.I. No.*	*Variety*	*C.I. No.*
Blackhull	6251	Mindum	5296
Bluejacket	12502	Nebred	10094
Bowie	13146	Pawnee	11669
Cheyenne	8885	Ponca	12128
Clarkan	8858	Quanah	12145
Comanche	11673	Red Chief	12109
Concho	12517	Red Jacket	12713
Early Blackhull	8856	Sioux	12142
Kanking	12719	Stafford	12706
Kanqueen	12762	Termarq	6936
Kharkof	2208	Triumph	12132
Kiowa	12133	Turkey	1558
Chiefkan-Oro X Tenmarq	12518	Westar	12110
		Wichita	11952

Except for Mindum, a spring durum, all varieties listed are winter wheats. With most varieties each transfer of mites was made to plants in two pots. The plants were infested with viruliferous mites 14 days after planting, when nearly all of them were in the three-leaf stage. Microscopic examinations of entire plants were made 2 and 3 weeks later. All the varieties were highly satisfactory for mite development, more so than any of the wild grasses. The mites transmitted the wheat streak-mosaic to every host plant.

Some observations were made on the increase of the mites on maturing wheat. Two varieties, Wichita and Pawnee, vernalized and growing in pots in the greenhouse, were heavily infested when they were in the boot stage, but there was little increase in population on either variety. A small number of Pawnee plants that had been vernalized were grown in pots in the greenhouse until jointing occurred, and then infested with the mites. Populations on these plants were never so heavy as on the non-vernalized plants. Apparently the plant is less favorable as a host after jointing than in the seedling stage, perhaps because of its rapid growth and approaching maturity.

Barley.—The following varieties of winter barley were included in these studies: Dicktoo, C.I. 5529; Kentucky No. 1, C.I. 6050; Ludwig, C.I. 7525; Poland, C.I. 6280; Pueblo, C.I. 8070; and Reno, C.I. 6561. The barley plants were handled in the same manner as the wheats, but were infested at various times. Viruliferous mites were used in infesting all varieties except Dicktoo, and each test was replicated twice.

All these varieties were favorable hosts for the mite. Barley was possibly a better host than wheat. The wheat streak-mosaic was transmitted to each of the varieties tested for transmission. No data were obtained on the effect of jointing or maturity of barleys. Mites transferred from diseased barley to wheat transmitted the disease to the wheat.

Oats.—The following oat varieties were included in the studies:

Variety	*C.I. No.*	*Variety*	*C.I. No.*
Ajax	4157	Fulton	3327
Alamo	5371	Kanota	839
Andrew	4170	Mo. 205	4988
Bond	2733	Nemaha	4301
Cherokee	3846	Osage	3991
Clintafe	5869	Richland	787
Clintland	6701	Santa Fe	4518
Clinton	3971	Victoria	2401

The plants were grown in the same manner as the wheat plants. The oats were infested 14 days after planting, and microscopic examinations of entire plants were made 10 days and 3 weeks later. Osage was the only variety showing any increase of the mite, and it was very poor. Mites occurred on all varieties for several days after infestation and caused the typical rolled-leaf condition. Viruliferous mites were able to transmit the disease to all the varieties on which they were used—Bond, Cherokee, Fulton, Kanota, Richland, Santa Fe, and Victoria. Nonviruliferous mites cultured on diseased oats for 2 to 3 days could carry the disease back to wheat.

Sorghum.—Twelve varieties of sorghum were tested. Two replicates of each variety were infested with mites 18 days after planting and a third replicate after 28 days. Since sorghums have been reported to be immune to the wheat streak-mosaic virus when manually inoculated, mites from diseased wheat plants were used to check on possible transmission by mites. None of the test plants became diseased, and mites transferred back to wheat from sorghum at various times did not transmit the disease to the wheat. Microscopic examinations of entire plants were made 2 and 3 weeks after infestation. The results of the tests on sorghum are shown below:

Variety	*Increase of mites*
Atlas	Poor-fair
Club X Westland	Good
Comb. 60	Good
Early Hegari	Fair-good
Ellis	Poor-fair
Martin	Poor-fair
Midland	Poor-fair
Miloca 56	Fair
Sorghum almum	Poor
Sorghum versicolor	Good
Westland	Good-very good
White Westland	Very good

All the varieties tested were suitable for reproduction of the mite, although to varying degrees. Westland and particularly White Westland had much larger colonies than any of the other varieties. On sorghum, as on the other hosts tested, the mite populations decreased as the plant matured. However, in most of the sorghums the decrease was more pronounced. The plants were easily infested as seedlings and supported large populations, but when infested after internodes appeared the populations were very small and confined mainly to the whorl. As in the other plants studied, the characteristic rolling and trapping of leaves occurred when the plants were infested in the seedling stage.

Sudan grass.—The following varieties of Sudan grass were tested as hosts of *Aceria tulipae:* Greenleaf, K2, Sweet Sudan 372, Taft, and Wheeler. The plants were infested with nonviruliferous mites 12 to 14 days after planting, and microscopic examinations of entire plants were made 2 and 3 weeks later. All the varieties tested were suitable as hosts, but none were very good and two were poor. As in all the other plants on which the mite was able to survive, the leaves were rolled and trapped. Also the mite population decreased as the plant matured. No records were made of mite transmission of the streak-mosaic to this host.

Corn.—Ten varieties of corn were tested in the same manner as the sorghums. The plants were infested 10 to 12 days after planting. The mites were taken from cultures containing both healthy and diseased plants, but no records were kept on the transmission of wheat streak-mosaic. Entire plants were examined for mites 2 and 3 weeks after infestation. The increase on the different varieties is shown below:

Variety	*Increase of mites*	*Variety*	*Increase of mites*
Country Gentleman sweet corn	Fair	K-1639	Poor
Dakota White	Fair	K-1830	Poor-fair
DD-F_2	Poor	K-2234	Fair
Falconer	Poor-fair	Midland	Poor-fair
Golden Giant sweet corn	Poor-fair	Pride of Saline	Poor-fair

All the corn varieties served as host plants, but none were very satisfactory. As with the sorghums, the mites were much more numerous on very small plants. After the appearance of internodes, the colonies practically disappeared and it was difficult to reinfest the plant. The characteristic rolling and trapping of the leaves was common in heavily infested small plants. As the plants grew older, leaves that had had heavy populations when younger were flecked with small white scars, apparently caused by the feeding of the mites.

DISCUSSION.—This investigation indicates that one or more of several common grasses or crop plants could be hosts to the wheat curl mite from May to September. Western wheatgrass is the only plant other than wheat on which the mite has been found reproducing in the field. However, the presence of diseased green foxtail, jointed goatgrass, sandbur, lovegrass, downy chess, ticklegrass, and ryegrass in the field in the last three summers indicates that they have been fed upon by the mite.

It is possible that the mites move around on the various grasses available between harvest time and the emergence of volunteer wheat. Survival of the few mites that reach the volunteer wheat appears to be sufficient to give rise to an infestation.

Corn and sorghum are perhaps the major crop plants that are available after the wheat harvest and before the emergence of volunteer wheat. No mites have yet been observed on these plants in the field, and it is probable that, if present on these plants, the population would be very low. Apparently the mites thrive best on plants having leaves that are easily rolled by their feeding, thus providing protection. Plants with narrow leaves, such as wheat, barley, and some of the wild grasses, might therefore be more attractive than corn or sorghum.

SUMMARY.—A study of the host range of the wheat curl mite, *Aceria tulipae* (K.), was made during the winter of 1953–4 in the greenhouses at Manhattan, Kan. The plants tested included 24 species of wild grasses, 27 varieties of wheat, 6 varieties of barley, 16 varieties of oats, 12 varieties of sorghum, 10 varieties of corn, and 5 varieties of Sudan grass.

The mite was able to reproduce on all tested varieties of wheat, barley, corn, sorghum, and Sudan grass, and on 12 of the wild grasses. The degree to which the populations increased varied considerably. Wheat and barley appeared to be the best hosts.

Wheat streak-mosaic was transmitted by the mite from wheat to all of the wheat and most of the barley varieties, and to varieties of oats and species of wild grasses.

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Effectiveness of Milky Disease in Controlling Japanese Beetle in Ohio[1]

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A bacterial disease, *Bacillus popilliae* Dutky, commonly known as the milky disease of the Japanese beetle larvae, was first introduced into Ohio in 1941. This disease was found early in the Japanese beetle investigations in New Jersey where it was reported to have affected as many as 50 per cent of the larvae in the field. In that area it was thought to have had a significant part in the ultimate solution of the Japanese beetle problem. Shortly after its discovery, a procedure was worked out whereby it was possible to propagate the disease organism in the laboratory and transfer it to the field (White & Dutky 1940). Several investigators including White (1941), Dutky (1941), and Langford *et al.* (1941) have reported upon the effectiveness of this disease when the organism was distributed manually in areas infested with Japanese beetle larvae. The propagation procedure was eventually presented to the different states that were confronted with the Japanese beetle problem.

The spore dust was first distributed in Cleveland and Youngstown, Ohio, by representatives of the Federal Bureau of Entomology and Plant Quarantine. At that time the supply was limited because the laboratory at Moorestown, New Jersey, could not produce the dust in sufficient quantities to supply the demands. Accordingly the Ohio Agricultural Experiment Station initiated a program for propagation of the spore dust in order to continue the distribution in the more heavily infested areas in the state.

During the period from 1941 to 1945, 2104 pounds of spore dust were produced in Ohio. This material and that furnished intermittently by the Bureau of Entomology and Plant Quarantine was distributed during the period 1941 to 1949. The data in table 1 show the amount of spore dust distributed in the various counties and the number of locations covered with the material.

The spore dust was distributed at different rates in areas approximately 2000 feet apart each way throughout the infested areas. Distribution was accomplished with a rotary hand corn planter with the mechanism set wide open. This would permit approximately 2 grams of the spore dust, standardized at 100 million disease spores per gram, to be dropped at each spot on the ground. The rate of application varied with the interval between the spots. For example, when the spore dust was distributed at 10-foot intervals, $1\frac{3}{4}$ pounds would be used per acre. When the interval was $3\frac{1}{2}$ feet, 7 pounds would be used per acre.

It may be noted from table 1 that Washington County is not listed as one of the counties in which milky disease spores were distributed. Yet in subsequent tables this county is shown to have had a high incidence of milky disease.

In the fall of 1949 it was reported that turf in the Marietta Country Club was being damaged by Japanese beetle larvae and a survey was conducted to determine the density of larval population. During this survey

Table 1.—Amounts of spore dust distributed in the different counties in Ohio.

COUNTY	POUNDS OF SPORE DUST	NUMBER OF LOCATIONS
Ashland	70.0	several[a]
Columbiana	7.5	1
Cuyahoga	1317.75	485
Gallia	52.5	several[a]
Guernsey	239.0	58
Huron	140.0	several[a]
Jefferson	126.75	2
Lake	120.75	26
Licking	72.75	7
Mahoning	431.25	95
Trumbull	50.5	7
Wayne	3.5	1

[a] In these instances the spore dust was distributed by representatives of the State Department of Agriculture in gardens where the turf was treated with insecticides. The definite number of locations was not noted.

[1] Accepted for publication June 9 1955.

Table 2.—**Average per cent infection of the Japanese beetle larvae for all sampling dates by years for the different counties.**

County	Per Cent Larval Infection										
	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954
Cuyahoga	4.9	8.8	1.6	.7	17.2	14.4	6.1	20.0	11.4		
Columbiana						.6	1.1	3.8	3.0	1.4	1.0
Guernsey		16.3	.4		2.0	7.4	7.4	19.5			
Lake		37.5	.4			4.9		3.3			8.1
Licking			.5								
Mahoning		17.9	.0	.4		6.3	2.9	15.5			
Jefferson							0.0	2.0	10.0	9.8	
Trumbull								1.3			
Washington						7.8	23.9	34.7	5.5	9.0	2.7

several of the grubs were found infected with spores of milky disease. The occurrence of diseased larvae was considered remarkable because spore dust had not been distributed in the area or within a distance of 70 miles from the golf course. In the first survey, 153 Japanese beetle larvae were found in 38 samples. Of this number 12 or 7.8 per cent of the larvae were found diseased. In 1950, diseased larvae were found only in the golf course and in an adjacent pasture. During the 1951 fall larval survey, diseased larvae were found in increasing numbers up to 5 miles from the golf course. At the end of the 1954 season, diseased larvae were found in all areas that averaged more than 5 larvae per square foot.

It is not known how the milky disease organism reached the Washington County area. However, it is suspected that birds were responsible inasmuch as White (1941) states "experimental studies have shown that milky diseases may be spread by birds." Diseased Japanese beetle larvae were submitted to Dutky and the disease producing organism was identified as *Bacillus popilliae* Dutky.

Although the milky disease spore dust was being distributed in small quantities from 1941 on, a detailed survey in an attempt to determine its effectiveness was not started until the fall of 1944. Prior to this time an occasional milky diseased grub was found in the specimens taken in seasonal history studies.

Surveys were conducted by examining 100 larvae or those found in ten ½-square-foot samples taken at random from or adjacent to turf areas treated with spores of the disease. The larvae found in a general larval survey in the infested areas were also used in determining the extent of larval infection. The data in table 2 show the results of the surveys for the different counties for the various years.

The data shown for the different years, except for 1946, 1947, and 1948, were obtained by taking the average of the accumulated weekly records for the months of August, September, and October and again for May and June of the succeeding year. Data for 1946, 1947, and 1948 were obtained by taking samples at the various locations during the first week in June.

In reviewing the data in table 2, it is readily noted that the per cent of infected larvae taken at any one location varied from year to year. These differences are probably due in part to the variation in the time at which the samples were taken. The sampling records for 1945, not shown here in detail, indicated that the period of greatest incidence occurred during the first week in June. However, when the different areas were sampled during the first week in June in 1946, the infection rate was found to be much lower than that found in 1945. The seasonal history studies in Forest Hill Park in 1946 indicated that if the milky disease samples had been taken approximately a month later, the per cent of infected larvae would have been much greater for Cuyahoga County. At that time as many as 50 per cent of the 1945–1946 larvae were found infected. However, in averaging the weekly seasonal records, it was found that the average per cent of infection for all samples was only 4.6 for the location.

The data presented in table 2 do not indicate the true importance of milky disease as a natural control agency of the Japanese beetle larvae. These data show only the average per cent of infected larvae for a single sampling date or the average of several sampling dates for the various locations in the state. When the differences in population between early and late fall and early and late spring or from early fall to the following late spring are compared, the true value of milky disease as a control agency can be seen. The data in tables 3 and 4 show a

Table 3.—**Population per square foot of immature stages of the Japanese beetle in the Bratenahl Golf Course, Cuyahoga County, for different months of the year.**

Year	Month	Population per Square Foot		Brood
		Treated Area[a]	Untreated Area[b]	
1942	April	32.4	26.8	1941–1942
	June	6.0	28.0	1941–1942
	August	72.0	97.2	1942–1943
	October	43.2	77.2	1942–1943
1943	April	45.9	69.3	1942–1943
	June	34.2	41.1	1942–1943
	August	27.0	75.0	1943–1944
	October	10.5	49.2	1943–1944
1944	April	16.2	44.4	1943–1944
	June	6.3	34.4	1943–1944
	August	6.6	28.2	1944–1945
	October	1.5	7.8	1944–1945

[a] Population in area treated with spore dust in 1941.
[b] Population in similar area untreated, 500 feet from[a].

Table 4.—Population of Japanese beetle larvae in various locations in Ohio at different times.

Location	Month	Larvae per Square Foot[a]	Per Cent Reduction in Numbers	Average Per Cent of Larvae With Milky Disease[b]
Cuyahoga County Forest Hill Park	May	21.4		2.9
	June	7.2	66.3	40.0
	Aug.	47.1		2.0
	Sept.	28.8	38.9	35.2
Cuyahoga County Riverside Cemetery	May	18.6		10.0
	June	3.0	83.9	22.0
	Aug.	16.5		22.6
	Sept.	11.7	29.7	45.1
Cuyahoga County Washington Park	May	14.1		.0
	June	7.2	48.9	23.2
	Aug.	18.8		2.9
	Sept.	14.7	21.8	21.5
Columbiana County Springhill Cemetery	May	23.7		.0
	June	15.0	36.7	3.8
	Aug.	35.1		.9
	Sept.	28.5	18.8	4.0
	June	6.3	77.9	.0 (1952)
Guernsey County McCollough Farm	May	26.7		6.7
	June	8.1	66.7	23.5
	Aug.	3.3		.0
	Sept.	4.8	.0	.0
Jefferson County Belleview Golf Course[c]	May	45.0		.01
	June	43.2	4.0	.0
	Aug.	55.8		.0
	Sept.	51.0	8.6	.0
Washington County Marietta Country Club	May	28.5		15.3
	June	3.0	89.5	57.7
	Aug.	24.3		.0
	Sept.	6.9	71.6	14.8
	Aug.	15.3		5.6 (1954
	Oct.	2.4	84.3	.0 (1954

[a] Includes only larvae not diseased.
[b] Data were taken in 1951 unless otherwise indicated.
[c] Located approximately ½ mile from the nearest milky disease distribution point.

reduction in larval population much of which can be attributed to the effectiveness of milky disease.

The data in table 3 show a marked reduction in population per square foot from April to June and from August to October in the area treated with spore dust. The population in the untreated area on the other hand remained at a much higher level throughout the sampling period.

In table 4 is shown the population of the 1950–1951 brood in May and June of 1951 and the 1951–1952 brood in August and September of 1951 for a number of areas and in June of 1952 for the Springhill Cemetery in Columbiana County.

With but two exceptions, the data in table 4 show an increase in the percentage of diseased grubs and a corresponding decrease in grub population between sampling dates in May and June, August and September, and August and the following June. The exceptions are for the Belleview Golf Course in Jefferson County and for the August to September period on the McCullough Farm in Guernsey County. Since the Belleview Golf Course is located about ½ mile from the area treated with spores in 1949, and about 3 miles from the area treated in 1942, it is probable that the low infection rate and low larval reduction are due to a slow transfer of the spores in that area. In the Guernsey County area high temperatures and low rainfall during July and August are undoubtedly responsible for the results obtained for the August to September record.

When the differences in population are computed from one sampling date to another, the effectiveness of the milky disease becomes more apparent. These data indicate that milky disease is playing an important role in reducing the grub population in most of the areas under observation. It seems to be exceptionally effective at the Marietta Country Club.

Summary.—The data presented show a marked fluctuation in the incidence of diseased Japanese beetle larvae from locality to locality, from month to month and from year to year. However, the marked decrease in larval population from August to the following spring in diseased areas is a real indication of the value of the disease in holding Japanese beetle infestations at a low level. The occurrence of the disease organism in Washington County, a distance of at least 70 miles from any release point, indicates that it will not be necessary to distribute the organism manually in all newly infested areas.

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ANNUAL REVIEW OF ENTOMOLOGY

VOLUME I

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Aseptic Rearing of the Pink Bollworm on Synthetic Media[1]

ERMA S. VANDERZANT[2,3] and RAYMOND REISER[3]

Although phytophagous insects cause tremendous economic losses in our farm crops, their biochemistry has not been extensively studied. Most studies of these destructive insects have been made on their susceptibility to insecticides or on other means of control. It is reasonable to believe, however, that an understanding of the nutrition and metabolism of phytophagous insects may lead to new and more fundamental methods of control. The development of an adequate synthetic diet is a prerequisite to the study of insect metabolism under controlled laboratory conditions.

Many workers have reported the rearing of insects on artificial diets in the laboratory. However, in many of these studies microorganisms were not excluded; hence their contributions to the diets cannot be estimated. Delcourt & Guyénot (1911) were the first to rear an insect, *Drosophila*, under aseptic conditions. The nutritional requirements of several other insects similarly reared have since been defined and reviewed by Trager (1953). Friend (1954), however, is the only worker who has reported the rearing of a phytophagous insect, the onion maggot, *Hylemya antiqua* (Meig.), on chemically defined diets under aseptic conditions.

Little is known about the nutritional requirements of the phytophagous Lepidoptera. Bottger (1942) was the first to rear a representative of this group, the European corn borer, *Pyrausta nubilalis* (Hbn.), on an artificial diet. Beck (1949, 1950) also reared this insect in the laboratory and used aseptic conditions. Ishii (1952, 1954) adapted Beck's method to the rearing of the rice stem borer, *Chilo simplex* (Butler), and Matsumoto (1953, 1954) has done similar work with the oriental fruit moth, *Grapholitha molesta* (Busck). Several years ago studies on the pink bollworm, *Pectinophora gossypiella* (Saund.), were begun in our laboratory, and a preliminary paper by Beckman *et al.* (1953) reported the possibility of rearing this insect on synthetic media. Since that time many modifications have been made and aseptic rearing technique has been introduced. These improvements are reported in the present paper.

EXPERIMENTAL PROCEDURES.—*Preparation of aseptic eggs.*—Moths emerging from cotton-plant debris were used as a source of eggs. These moths were placed in an oviposition box 8×8×8 inches which had an opening on one side covered by a double screen. The box was waxed on the inside to prevent oviposition on the walls. Sugar water in a ball of cotton was provided as food. The box was cleaned frequently with detergent solution to prevent the growth of molds. A washed cotton leaf was placed on the screen and then covered with cellophane.

The moths deposited their eggs both on the leaves and on the cellophane. The eggs were removed with a sable brush and transferred to a solution containing 0.25 gram of mercuric chloride, 6.5 grams of sodium chloride, 0.5 gram of sodium glycocholate, 1.25 ml. of concentrated hydrochloric acid, 250 ml. of ethyl alcohol, and 750 ml. of distilled water. After remaining in this solution for 20 minutes, they were washed twice with sterile distilled water. Special glass tubes similar to test tubes with openings at the bottom were fitted with rubber tubing which could be closed with pinch clamps, and eggs could be transferred from one tube to another by opening the pinch clamp. The solution and the equipment used for this procedure have been described by Stride (1953). The washed eggs were collected in a sterile vial on a filter paper placed above absorbent cotton, which removed the excess water.

In early experiments, eggs were examined for contaminating microorganisms by plating portions of eggs on standard nutrient agar and on potato-dextrose agar. The plates were incubated at 27° C. and examined after 2 and 5 days, respectively. In subsequent experiments, absence of contamination in the rearing vials was assumed to be proof that the eggs were free of microorganisms.

Preparation of media.—The rearing media were based upon the albumen medium used by Beckman *et al.* (1953). Albumen was used because it contains all the amino acids ordinarily found in proteins, is soluble in water and hence mixes readily with the other ingredients, and coagulates upon heating to give a solid consistency upon which the larvae can feed. The compositions of the media are given in table 1.

The composition of the vitamin mixture per 100 grams of medium was as follows:

Vitamin	*Milligrams*
Thiamin	0.60
Riboflavin	0.90
Calcium pantothenate	2.00
Niacin	6.25
Pyridoxin	0.80
Folic acid	0.125
Biotin	0.018
p-Aminobenzoic acid	1.50
Vitamin B_{12}	0.005

An aqueous solution of vitamins was prepared so that 1 ml. contained these amounts.

The dry ingredients and fat were weighed out and mixed with the choline chloride solution (0.1 gram per milliliter of water), vitamin solution, and water in a Waring Blendor until homogeneous. A stabilizing agent, either sodium carboxymethylcellulose (CMC, Hercules Powder Company) or alginic acid (Kelco Company), was then added and blended with the other ingredients. The acidity was then adjusted to approximately pH 6 with 2 M potassium hydroxide.

Preparation and inoculation of vials.—Approximately 3-ml. portions of medium were placed in 17×60 mm. plain vials plugged with nonabsorbent cotton or vials with Bakelite screw caps. The vials were then autoclaved at 15 pounds pressure for 25 minutes, and allowed to stand at room temperature for 1 to 2 days until excess moisture had evaporated. They were then ready for inoculation.

In the first experiment (Medium 1, Table 2), five eggs were transferred from the collection vial to each vial

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Table 1.—Composition of nutrient media for growing the pink bollworm.[a]

Constituent	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10
Egg albumen	8.0	8.0	8.0	8.0	8.0	11.5	15.0	7.5	12.0	7.5
Glucose	8.0	8.0	8.0	8.0	8.0	8.0	2.5	7.5	2.0	7.5
Sucrose	—	—	—	—	—	—	1.25	2.5	2.0	2.5
Corn oil (Mazola)	6.0	6.0	6.0	3.0	3.0	3.5	0.3	1.25	10.0	0.3
Cholesterol	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.5	0.3
Wesson's salts or salts 4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.6	1.0
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.13	0.13	0.20	0.13
Inositol	0.1	0.1	0.1	0.1	0.1	0.1	0.07	0.07	0.10	0.07
Lecithin (animal)	1.0	1.0	1.0	1.0	1.0	—	—	—	—	—
Cotton leaf meal	—	—	0.6	0.6	—	—	—	—	—	—
Ribose nucleic acid	—	0.3	0.6	0.6	0.6	—	—	—	—	—
Sodium carboxymethylcellulose	2.5	2.5	2.5	2.5	3.0	—	—	—	—	—
Alginic acid	—	—	—	—	—	2.0	1.45	1.75	1.60	1.75
Cellulose	—	—	—	—	—	—	3.0	3.0	10.0	3.0
Dried forage juice	—	—	—	—	—	1.0	—	—	—	—
Vitamin mixture	(As described in text; same for all media)									
Water	73.0	73.0	72.0	75.0	75.0	73.0	75.0	75.0	60.0	76.0

[a] Figures in grams.

with sterile sable brushes. As a result of cannibalism, only one larva was found in each vial at the end of the experiment. Subsequently only one newly hatched larva was similarly placed in each vial.

Use of vials closed with screw caps.—The vials with screw caps were of the same size as those in which cotton plugs were used and were prepared in the same manner. In order to eliminate free moisture, either the vial walls above the media were flamed or small rolls of dry, sterile filter paper were placed inside. The larvae were placed aseptically in the vials and the caps put on tightly. After the larvae reached the fourth instar, the caps were loosened about one-quarter turn to permit slow exchange of air with little loss of moisture. If a vial became moldy after the larva had stopped feeding, the larva was transferred to a dry vial; otherwise, it was left in contact with its food until it pupated. The usual procedure as described below was then followed.

Observations on development.—Observations on and records of the growth of the larvae were made at frequent intervals. Dates on which pupation occurred were recorded. The pupae were then removed from the rearing vials, examined, and weighed individually. When moths emerged, they were placed in oviposition boxes as previously described.

RESULTS AND DISCUSSION.—Data for the development of the pink bollworm reared aseptically on various synthetic media are given in table 2. On the first five media a large number of larvae died in the first instar. Some of the larvae died after becoming lost in the cotton plugs. In most cases, however, death occurred by drowning in water condensed on the sides of the vials, or by becoming trapped in the fat, which did not mix well.

Table 2.—Growth of the pink bollworm on synthetic media.

Medium	Number of Vials		Number of Larvae Remaining in Instar		Number of Pupae	Number of Adults	Minimum Larval Period (Days)	Average Pupal Weight (Milligrams)
	Cotton Plugs	Screw Caps	Second and Third	Fourth				
1	109	—	6	40	32	22	30	—
2	42	—	2	5	10	8	30	—
3	80	—	14	10	20	20	29	—
4	80	—	11	10	40	40	29	—
5	120	—	66	16	10	9	35	—
6	30	—	7	1	22	22	24	13.3
		18	—	1	17	16	24	12.2
7	58	—	38	3	12	11	35	6.5
		20	9	—	11	10	31	6.5
8	66	—	13	4	47	45	28	9.0
		46	—	5	41	38	24	11.5
9	34	—	24	—	1	—	—	—
		15	2	—	13	8	25	11.2
10	—	15	4	—	11	10	33	11.4

Since the squares and bolls upon which the larvae feed contain from 75 to 85 per cent of water, it is necessary to have some substance present which absorbs water and also keeps the fat homogeneously mixed. Furthermore, the larvae appear to be quite sensitive to the texture of the medium, because they feed more readily on a hard, firm medium than on one which is gelatinous. In the first experiments sodium carboxymethylcellulose was used to thicken and stabilize the media. However, rather large amounts were necessary to incorporate both the fat and the water. Subsequently it was replaced by a derivative of seaweed, alginic acid, which gave a better texture to the medium and could be used in smaller amounts. An attempt was made also to use larger amounts of albumen, which thickens upon heating, forming a solid medium, but the growth of the larvae was retarded. This was probably due to the imbalance of nutrients caused by increasing the protein.

In the cotton-plugged vials many larvae failed to reach the fourth instar, and many that did failed to pupate. The results were variable, and it appeared that the drying of the medium was responsible for the retarded growth. Although a pan of water was kept in the incubator, constant humidity was difficult to maintain, and the medium became quite dry during the approximately 30-day period required for the growth of the larvae. A constant humidity of 76 per cent, achieved by keeping the vials in a closed container over saturated sodium chloride solution, was found to be favorable. For best results the water content of the medium should be 70 to 80 per cent. Differences in larval development on dry and moist media were observed in media 6 to 9, where vials closed with screw caps to keep the moisture constant were used concurrently with cotton-plugged vials.

On all synthetic media the larval period was longer than that of larvae developing on either squares or bolls.

Fenton & Owen (1932) reported larval periods of 14 days on squares and 20 days on bolls under normal field conditions. Only 1 to 3 larvae in each group developed in the minimum periods given in table 2. Pupation in the majority of larvae extended over a 3- or 4-week period. At the end of this time larvae that remained were in the resting stage and no further records were kept on their development. Media 1 to 5 produced a large number of resting larvae. Beginning with medium 6 the resting stage was terminated by covering the larvae with wet cotton in a Petri dish. This accounts for the small number of fourth-instar larvae remaining in subsequent experiments. Larvae shown in table 2 as remaining in the second and third instars eventually died at these stages.

Average pupal weights were similar for most media but lower than weights of normal field specimens. The range of pupal weights was 6.0 to 20 mg.

The pink bollworm can develop in spite of wide variations in the dietary constituents. On most media almost all larvae that pupated produced normal adults which deposited fertile eggs. Abnormal pupae appeared in several cases, but only on medium 8 were there a striking number of abnormalities. These pupae died in the middle of the pupal period, and had large black spots about the head, eyes, and thorax. Several moths that emerged could not fly; others appeared to be normal. This medium had a rancid odor at the end of the experiment, and abnormalities may have been due to some toxic product of the oxidized fat.

Several constituents of the medium did not appear to contribute to its nutritive value. If the pink bollworm has no cellulases or other polysaccharases, in agreement with the findings of Swingle (1928), Beck (1949), and Yushima & Ishii (1952) for other phytophagous Lepidoptera, it can be assumed that sodium carboxymethylcellulose, alginic acid, and cellulose have no nutritive value other than, perhaps, traces of inorganic substances. Lecithin was added as an aid in incorporating the fat, but was later omitted because the stabilizers can serve this purpose as well. Ribose nucleic acid did not appear to have a stimulatory effect. It is doubtful whether the addition of inositol was necessary (Trager 1953), since it has not been proved to be an essential nutrient for most other insects. Wesson's salts and salts 4 (Beckman *et al.* 1953) permitted equally good growth.

Beck (1953) found that aqueous plant extracts had to be added to the diet of the European corn borer to obtain optimum growth of this insect. Ishii (1952) and Matsumoto (1954) added similar plant extracts to the diets of the rice stem borer and oriental fruit moth, respectively. Therefore, it was believed that cotton leaf meal (dehydrated, ground cotton leaves) might be needed in the diet of the pink bollworm. However, no stimulatory effect upon the growth of the larvae was produced, nor did the cotton leaf meal make the medium more attractive to them. Dried forage juice (Cerophyl Laboratories) was used also, with similar results. A major difference between the diets for the pink bollworm and the diets used by these workers is in the kind of protein used. It may be that the albumen supplies some factor not present in the casein. If this is not the case, the nutritional requirements of the pink bollworm may differ from these insects in that an unidentified plant factor is not required.

It has already been pointed out that many pink bollworm larvae go into the resting stage, or diapause. It is therefore difficult to determine accurately the rate of development and to study the complete life cycle without interrupting the resting stage. Several theories for the diapause of the pink bollworm have been proposed. According to Ballou in Egypt (1920), nutrition is responsible. Squire (1940) showed that the number of larvae that go into the diapause increases as the water content of the food decreases. Fife's studies (1949) show that in Puerto Rico the diapause occurs during drought at any time of year, the moisture content of the resting larvae being lower than that of nonresting larvae. Taylor (1936) reports that diapause is induced by a number of factors which cause drying of the larvae or their food. The maturing of the seed, low humidities, lack of rainfall, and high temperatures during dry periods all contribute to induction of diapause. Although the evidence is in favor of environmental rather than nutritional conditions, it cannot be overlooked that maturing seeds contain less moisture and more stored carbohydrate and fat than young seeds. Other nutrients vary as well. Since diapause is a means of survival under unfavorable conditions, such as are found in winter when no food is available, it is reasonable to expect that insects in this state will differ in chemical composition from nonresting insects. These differences may depend on the composition of the food that has been consumed.

It is well known (Fenton & Owen 1932) that the length of the larval feeding period, the size of the larvae, and the number of resting larvae are different for larvae feeding on squares than for those feeding on bolls, and also differ with the age of the squares or bolls. The composition of the insect's food, particularly the protein, carbohydrate, and fat, changes considerably during the growth of the cotton plant. Media 7, 8, and 9 were therefore prepared to contain these nutrients in the same proportions as in bolls of different ages, on the basis of data presented by Bailey (1948). Medium 7 represents the ovum just after flowering, medium 8 the seed or intermediate age, and medium 9 the seed that is almost mature. Cellulose was added to give these media a more rigid structure, and the water content was decreased in medium 9 to compare with the maturing seed and also because of the high content of fat, which is not compatible with the water. Medium 10 was the same as medium 8 except for the fat content, which was the same as in medium 7.

Medium 7 produced small, slowly developing larvae, perhaps because of its high protein and low carbohydrate content. The effect of protein level and caloric intake upon insects has been reviewed by Haydak (1953). Low-calorie intake and high-protein diets have been found to retard the growth of several species, and the results with medium 7 may correlate with these findings. The small, slowly developing larvae apparently were not caused by the low fat content alone, because medium 10, which had the same low fat content, produced larvae similar to those reared on medium 8. The high fat content of medium 9 appeared to have no effect upon either larval size or diapause, because media 8 and 10 gave similar results. There appeared to be no correlation between these media and the number of resting larvae. This is not shown in

table 2, because the number of pupae includes those formed from resting larvae in which pupation was induced by the wet cotton treatment. There seems to be no effect of these media on the resting stage. However, the effect of diet upon diapause in the pink bollworm deserves more study, and other variations in the medium should be made.

These media contained, at least in small amounts, all the nutrients required by the pink bollworm to complete its life cycle, since viable eggs were obtained from the moths reared on them. Three generations have been reared on media to which no plant extracts were added. There is no reason to believe that many more generations could not be reared.

SUMMARY.—Techniques used for rearing the pink bollworm, *Pectinophora gossypiella*, on sterile synthetic media have been described. Of 259 adults obtained, many deposited fertile eggs. Three generations of pink bollworms were reared in media containing no plant extracts.

In a study of the effects of different protein-carbohydrate-fat ratios in the media upon the development of the pink bollworm, a high-protein diet was found to produce small, slowly developing larvae, but variations in carbohydrate and fat did not affect their size or growth rate.

The number of larvae in the diapause was not affected by changes in the protein, carbohydrate, and fat content of the media.

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PRICES OF REPRINTS

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Honey Bee Queens Whose Eggs All Fail to Hatch[1]

J. D. Hitchcock, *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*[2]

In May 1952 fifteen packages of honey bees, *Apis mellifera* L., with queens from a southern breeder were installed as colonies at Laramie, Wyoming. After 2 weeks four of the queens were found to be abnormal in that none of their eggs hatched. After another 2 weeks a fifth queen, identified by marking and clipping as the original queen in the package, laid only nonhatching eggs. Correspondence with the breeder revealed that three other customers in different States had complained of similar queens. One beekeeper reported 35 such queens out of 200, or an alarming 18 per cent. These queens appeared normal externally and laid large numbers of eggs in a regular manner as long as they were allowed to live. They usually laid only one egg per cell, depositing them upright on the base of each cell, just as normal queens do.

A count of the brood on one side of a comb from one of these abnormal package-bee colonies showed that there were 829 cells containing a single egg without any larval food, 23 cells containing two eggs of which one was shrunken, 9 cells with larval food and a shrunken larva, 5 cells with larval food only, and 1 cell containing an apparently normal larva about 3 days old. Thus only one egg out of 867 appeared capable of development, or about 0.1 per cent. Among the many eggs on the opposite side of the same comb was a single sealed cell which contained an apparently normal white-eyed worker pupa. Each abnormal colony had 2 to 3½ brood combs containing eggs only.

When frames of brood were exchanged between colonies, it was found that the package bees reared the eggs of a normal queen to maturity, whereas all the eggs from an abnormal package queen were cleaned out quickly by bees of a normal colony. Therefore, the failure of the package queen's eggs to hatch cannot be attributed to inability of the nurse workers to care for the eggs and hatching larvae.

Later this queen was introduced to a nucleus, and again all her eggs laid in worker comb failed to hatch. Then she was confined to a drone comb, but only a few of the eggs laid in drone cells hatched, and none were reared to sealing. Eggs from a normal queen were then given to the nucleus, and its bees were still capable of rearing most of them to sealing. They also transformed a few cells into queen cells, an indication that the workers realized they needed to supersede the abnormal queen. Since drones are produced parthenogenetically, and even eggs laid in drone cells failed to hatch, it seems probable that the sperms were not at fault, or at least were not the sole cause of the abnormality.

After all the normal brood was sealed and the queen cells were destroyed, the abnormal queen was allowed to lay in worker combs again. Then she was removed, leaving the nucleus queenless. Many queen cells were started, but not a single larva was reared. Even in dire necessity the workers were unable to rear brood from the abnormal queen's eggs. This queen laid nonhatching eggs for about 3½ months.

All five abnormal queens were dissected. Their ovaries were well developed, but all showed a yellowish or light-brownish spotting, principally near the base of each ovary where it joins the oviduct. The fat body in all queens also showed a brownish spotting. No microorganisms were discernbile in these spotted tissues. The spermathecae of all five queens appeared normal externally; two of them were crushed and contained abundant motile sperms. All other abdominal organs appeared normal. The blood appeared clear.

At first it was suspected that these queens might be in the early stages of some type of melanosis, perhaps caused by an unrecognized fungus, or might have a virus infection. A portion of a spotted fat body was macerated in saline and injected into the abdominal cavity of an anesthetized virgin. She survived at least 8 days, but was found dead on the tenth. Her dark-brown ovaries fell to pieces during dissection, whereas the intestinal tract was still whitish and firm, but his might represent post-mortem changes. Moreover, dissection of nine other living queens, including three failing queens, one drone layer, and five normal queens, showed all of them to have some spotting of the ovaries and fat body. Therefore, the spotted condition is not directly related to any parasitic infection.

Microscopic examination of the nonhatching eggs revealed definite development of the embryos, with considerable variation in the embryonic stages attained.

The breeder from whom these queens had been obtained reported in the spring of 1953 that he had changed the drone stock in his mating yards and apparently had eliminated such defective queens.

Literature.—Over 90 published records have been found of abnormal queens, all of whose eggs failed to hatch, but in view of the countless queens reared each year, and in line with statements of commerical beekeepers such as Miller (1906), who reported seeing only one such queen in 45 years, this abnormality must be considered rare.

The geographic distribution apparently is world-wide. Earliest records found for various countries are: Germany, Kleine (1861); United States, Marvin (1867); Switzerland, Halter (1881); Canada, Anonymous (1906); England, Betts (1924); and Czechoslovakia, Sutta (1953). The abnormality has also been described in the following countries, but perhaps solely from literature: Austria, Arnhart (1929); Moravia, Peterka (1940); and Brazil, Cury (1951). Fyg (1936) reviewed most of the significant literature.

Herlikofer (1864) exchanged pieces of comb containing eggs between colonies and so was the first to demonstrate that the abnormality is not caused by nurse bees neglecting or being unable to care for the brood. He also was certain, from the color markings of his queen and her progeny, that the same queen laid normal eggs in the autumn before laying nonhatching eggs the next spring.

Leuckart (1871) first demonstrated microscopically

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that such queens possess well-developed ovaries, that their spermathecae contain motile sperm, that some embryonic development occurs within the eggs, and that the chorion of the egg is of normal thickness.

Koschevnikov (1900) believed that a yellowish spotting of the fat body in normal queens is caused by an accumulation of waste products of catabolism in the oenocyte cells. He found such coloration especially abundant in old queens. Betts (1924) observed a case in which all worker eggs died but a few drones developed. However, she pointed out that they might have been the progeny of laying workers. Leuenberger (1926) beautifully photographed the embryonic development within nonhatching eggs, and (1928) observed a single well-formed pupa with pigmented eyes among many nonhatching eggs. Gooderham (1931), by occasionally adding bees to an otherwise dwindling colony, kept a queen whose eggs all failed to hatch laying continuously from early May to the middle of September. Isaacson (1932) described the recovery of a chilled and starved queen who thereafter laid mostly nonhatching eggs; the few that hatched and developed into workers only. Fyg (1934a), using biological stains on the reproductive organs of the queens and their nonhatching eggs, proved that no microscopically visible parasites were present.

Goetze (1935, 1936), in cross-breeding experiments with different strains of honey bees, observed the absence of an albino type expected from Mendelian ratios and found that some of the eggs failed to hatch. He concluded that certain genetic factors were lethal when homozygous, and suggested that this mechanism might also explain cases of completely "addled" eggs.

Fyg (1936) confined to drone combs two queens whose eggs all failed to hatch but their eggs laid in drone cells also failed to hatch. However, suspecting that even the eggs laid in drone cells might have been fertilized, he subjected one such queen to low temperatures—a procedure known frequently to cause normal queens to become drone layers. This queen then laid many eggs, a few of which did develop into drones. Fyg therefore concluded that the cause of nonhatching eggs occurs in the sperms, but he considered it some type of injury that prevented fertilization, rather than transmission of a lethal hereditary factor.

Mackensen (1951), in breeding experiments with artificially inseminated queens, postulated that the mortality in cases of low brood viability was correlated with inheritance of a series of haplo-viable homozygous lethal alleles similar in action to the sex alleles of *Habrobracon* (Whiting 1943). However, he did not observe any cases of complete egg sterility.

Roberts (1953), in Wisconsin by artificial insemination, mated the daughters of a certain queen with their own brothers, and then back-crossed four second-generation queens with drone sons of the original mother, whereupon two of these queens laid only nonhatching eggs. Another daughter of the original queen was out-crossed to drones of a cordovan strain, and one of her daughters was then back-crossed to drones of the original queen; then 10 of the third-generation queens were allowed to mate naturally, whereupon four of them laid only nonhatching eggs. Roberts therefore postulated that the original mother was heterozygous for a recessive mutation, which resulted in nonhatchability of all eggs laid by queens homozygous for the mutation.

DISCUSSION.—In most of the literature there is general agreement with the author's observations in regard to queens whose eggs all fail to hatch,—that (1) this abnormality is rare, (2) such queens lay large numbers of eggs in a regular manner for a long time, (3) their ovaries and fat body appear normal, (4) their spermathecae contain abundant motile sperm, (5) some embryonic development occurs, and(6) no microscopically visible parasites are present. Some differences of opinion are discussed below; most of them center around the question as to whether the cause of the abnormality is hereditary.

Phillips (1915) suggested that the chorion might be too thin, allowing excess evaporation, whereas Betts (1924, 1926) suggested that it might be too thick, either physically preventing the embryo from bursting it or hindering respiration. Leuchart (1871) was the only investigator actually to determine the chorion thickness, and he found it normal.

Arnhart (1929) described a parasitic melanosis of the ovaries caused by a yeast-like fungus, and suggested that cases of egg sterility were early stages of this disease in which the parasite was unrecognizable, but Fyg (1934 *a*, *b*) disproved this hypothesis. A single inoculation by the writer was inconclusive as to whether the spotted fat body contained an infectious virus. It seems doubtful that any parsite is involved, for then the abnormality would be expected to spread to other colonies, especially in large queen-rearing apiaries, or when brood combs are interchanged between colonies. Moreover, one would not expect queens to continue laying regularly for such long periods, if their reproductive organs were parasitized.

For all practical purposes the egg sterility of these abnormal queens is complete; yet careful observers have noted that an extremely small percentage of the eggs may hatch and be reared to sealing, and presumably reach maturity. Betts (1924) observed a very few drones, and Fyg (1936) obtained a few drones, but only after subjecting the queen to cold treatment. On the other hand, Leuenberger (1928) observed a single pupa with pigmented eyes, and it seems certain that this must have been a worker, for had it been a drone he surely would have said so. Isaacson (1932) observed that all the pupae reared in his case were workers. The writer observed a single pupa that definitely was a worker among approximately 1600 cells of nonhatching eggs. It seems to him that these cases of developing workers contradict the view of Fyg (1936) that the cause of nonhatching eggs lies solely in the sperms or their ability to effect fetilization. On the other hand, these few females might be produced parthenogenetically, for Mackensen (1943) showed that the occurrence of such females is widespread.

Isaacson's (1932) case might suggest that cold temperature is the cause, but Fyg's (1936) research and review of literature indicate that subjecting queens to cold usually causes them to lay drone eggs only, rather than nonhatchable eggs. Moreover, if cold temperature was the cause, one would expect the abnormality to occur much more frequently than it does.

The cross-breeding experiments of Goetze (1935, 1936) and Mackensen (1951), and especially those of

Roberts (1953) involving completely nonhatchable eggs, suggest that the abnormality is a hereditary one, perhaps a genetic mutation that is lethal only when it is homozygous. One of the principal objections to this view is that there seem to be definite records of certain queens that laid normal, hatchable eggs early in life but later laid only nonhatchable eggs. Among such records are those of Herlikofer (1864); McCarty (1911), who apparently observed a gradual decrease in the percentage hatchability; Anonymous (1922), in which the queen laid normally in the spring but suddenly began laying nonhatching eggs; and Krancher (1925), who reported a case similar to that of Herlikofer. Apparently none of these beekeepers identified their queens positively; yet it is unlikely that they would have been superseded without their owners eventually realizing it.

One such queen noted by the writer, which seemed to lay normal eggs at first but nonhatching eggs later, was positively identified, both by a spot of paint on her scutum and by having the wings on one side clipped short. Unfortunately, it is not absolutely certain that she laid normal eggs at first. The abnormality was not noticed in her package-bee colony until 2 weeks later than it was noticed in the four other packages installed at the same time. Young larvae were observed in her package colony, but possibly only a few such larvae, and possibly even these did not reach maturity.

These cases of queens that laid normal eggs first and only later began laying nonhatching eggs seem to disprove the view that the cause is genetic, for, if so, one would expect such queens to lay nonhatching eggs from the very start and throughout their life. However, it is possible that such queens mated with two drones of different genetic stock. Roberts (1944) showed that double matings are quite common on successive flights, and Taber (1954) presented data indicated that multiple matings may occur even during a single flight. Nevertheless, the normal sperm from one drone would have to be completely used up before the queen began utilizing the genetically defective sperm from the other drone. This would necessitate nonmixing of sperm in the spermatheca or a selective utilization from mixed sperm, and it is not known whether these occur in the honey bee. Of course, one also could assume that mutations or various genetically determined failures occurred in a normal queen herself, instead of being inherited from previous generations, in order to account for a change from the laying of hatchable to nonhatchable eggs.

The view that the cause is a genetic defect which must be inherited from both parents is sometimes objected to, on the grounds that all drones should then be reared normally, because they develop from unfertilized eggs according to Dzierzon's 1845 theory of parthenogenesis (Berlepsch 1861). Although it is commonly known that queens confined to drone comb may lay many fertilized eggs in drone cells, Fyg's (1936) view that none of the eggs laid in drone cells by these abnormal queens are unfertilized seems unlikely. Woodrow (1942), for example, observed 45 and 81 per cent of drones from two normal queens confined to drone comb. Unfortunately Fyg's paper was not seen by the writer until all his queens had been dissected; so the effect of cold treatment on a queen laying nonhatching eggs was not retested. Although it is certain that all eggs laid by these abnormal queens in drone comb fail to hatch, what percentage of these eggs are unfertilized, if any, remains unknown. Therefore, the percentage of drone progeny capable of development is unknown.

The fact that a queen breeder apparently eliminated such abnormal queens by changing his drone breeding stock suggests that the defect is hereditary; on the other hand, this result might have been purely coincidental. Certain cases of egg sterility in *Drosophila* are undoubtedly genetic (Dobzhansky 1941, see Chapter IX), but others simply are derangements resulting from supernumerary sperm (Huettner 1927). Egg sterility in other insects is so variable that it would be unwise to make any specific analogy with the honey bee. Possibly future cytologic or genetic studies may clear up the situation. For the present we simply do not know the true cause of complete egg sterility in the honey bee. It is possible that there are different types of such egg sterility, which have different causes.

SUMMARY.—Five queen honey bees, *Apis mellifera* L., were observed, and literature is reviewed on others, which laid large numbers of eggs in a regular manner but whose eggs all failed to hatch. Such records are rare, but cases have been reported from Germany, the United States, Switzerland, Canada, England, and Czechoslovakia.

Failure of the eggs to hatch is not caused by inability of the worker bees to care for the brood. Such queens have well-developed ovaries and their spermathecae contain abundant, motile sperms. Considerable embryonic development occurs within the eggs, but they fail to hatch even though supplied with brood food. The chorion is of normal thickness. There is a yellowish or light brownish spotting at the base of the ovaries and throughout the fat body, but this also occurs in normal queens. No microscopically visible parasites are present, and it is doubtful that a virus infection in involved. Such queens may continue laying regularly for more than 4 months. A very small percentage of eggs sometimes hatch but, contrary to the view of Fyg (1936), workers may develop from them, as well as drones. Some queens may lay hatchable eggs early in life, then later lay only nonhatching eggs. Eggs laid in drone cells also fail to hatch. The true cause of this abnormality remains unknown.

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Parathion, EPN, Dieldrin and Methoxychlor for Control of the Plum Curculio on Prunes[1]

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Of the numerous organic insecticides introduced during the past decade four have been accepted in varying degrees for plum curculio control. These are parathion and EPN of the thiophosphate group and dieldrin and methoxychlor representing the chlorinated hydrocarbons. Standard field tests do not reveal the factors accounting for the end results obtained with these materials. In an effort to obtain more specific information field tests have been supplemented with studies made under insectary and laboratory conditions. The factors evaluated were residual action, toxicity to stages present when treatments are customarily applied, and contact toxicity.

FIELD CONTROL STUDIES.—Tests were conducted using two schedules of timing, one a protectant program and the other eradicant. The protectant schedule was essentially that recommended to growers and was designed to prevent infestation by beetles invading host trees following emergence from hibernation. The second schedule directed against eggs, larvae and adults was adopted because under practical conditions sprays are often applied after some injury has occurred due to delay imposed by unfavorable weather or conflict with other spray treatments. Furthermore, ovicidal action may contribute to control by eliminating eggs deposited in the interval between sprays.

Sprays were applied with a standard hydraulic orchard sprayer operated at 400 lbs. pump pressure. Application was made from the ground using a single spray gun. Fruit and foliage were thoroughly sprayed to the point of run-off. Plots consisted of 9 trees each (3×3) and were replicated four times. Individual trees in each plot were selected as the test unit. All dropped fruits were collected at intervals of 5 days or less. Fruits collected from individual trees were placed in screen-bottom racks and held in the insectary. The mature larvae were recovered as they deserted the fruits and dropped into trays beneath the racks. Larvae were collected daily so as to disclose the rate of emergence as well as the total number of larvae recovered during the season.

Protectant Program.—The results of several seasons tests are summarized in table 1. All materials were effective under some seasonal conditions. Seasonal fluctuations were greatest for parathion and least for dieldrin. Dieldrin was the only material which was clearly adequate under a two-treatment program.

The results of the 1953 season are of particular interest because with the exception of dieldrin materials proved less effective than in other seasons despite a much lighter infestation. These results are in keeping with grower experience in that 1953 proved to be a "problem

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Table 1.—Summary of plum curculio control on prune.

Year	Dates of Application			Check Infestation (Larvae per Tree)	Per Cent Control			
					Parathion	EPN	Dieldrin	Methoxychlor
1949	5/16	5/26	6/5	1292	97.5	—	—	98.8
1950	6/5	6/15	6/26	790	97.2	—	—	—
1951	5/28	6/8	6/18	1493	99.3	99.1	—	93.1
1952	5/29	6/9	—	1381	90.2	—	98.9	—
1953	5/25	6/2	—	384	66.5	82.8	99.2	78.9
1954	5/26	6/4	—	499	89.1	—	—	—

year" for plum curculio control. This fact warrants more careful consideration of these results.

Sprays were applied at "shuck-split" stage, May 25 and again on June 2. The interval between sprays was shortened to 8 days as precipitation followed the first spray and undoubtedly reduced the residue deposit. The two-treatment program followed here was not expected to prove entirely adequate but was used to accentuate differences in materials. The rate of larval emergence is shown graphically in Fig. 1. Poor control was apparently due to lack of residual effectiveness as all materials initially gave good control judging by the larval emergence curves. The results reported for 1953 might be explained in part by the unusual weather which prevailed during the period of activity by the pest. Temperature during this period was well below normal. The mean daily temperature remained below the threshold of activity (60° F.) for the entire interval between the first and second spray. In addition, 0.9 inch of rain fell during this period. Under these conditions the control obtained was due largely to the second spray. Rainfall was not a factor in the effectiveness of this spray. Following the second application, temperature remained unfavorable for several days then rose well above the threshold. It was likely during this period and shortly thereafter that most of the injury occurred. Apparently residues had weathered below the effective limit before this time, except in the case of dieldrin. While these conditions

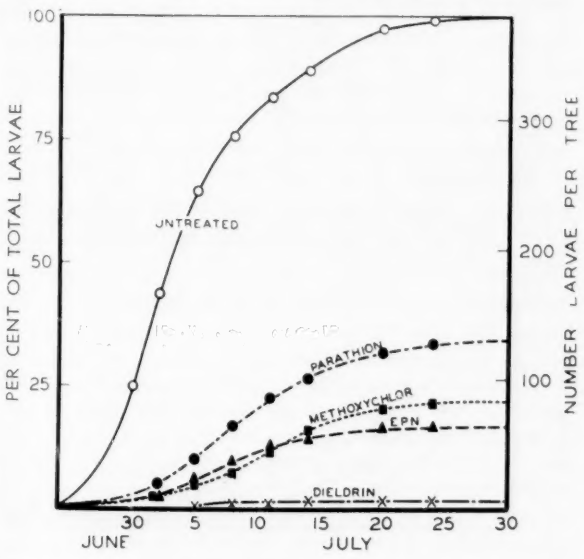

FIG. 1.—The accumulative emergence of plum curculio larvae from prune drops. Protectant schedule, sprays applied May 25 ("shuck-split") and June 2, 1953.

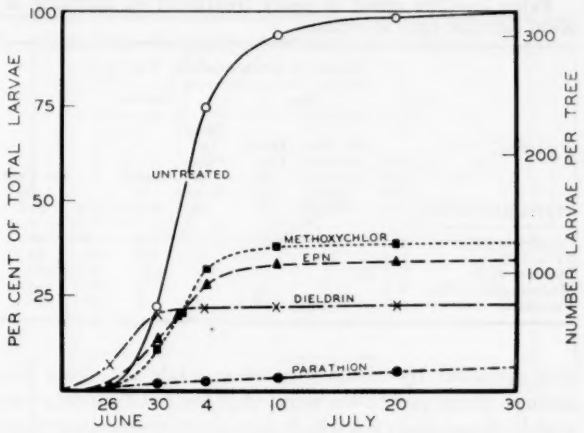

FIG. 2.—The accumulative emergence of plum curculio larvae from prune drops. Eradicant schedule, single spray applied June 11, 1953.

constitute a rather rigorous test the significant fact is that dieldrin was effective. The evidence at hand is insufficient to establish clearly the relationship between climatic conditions and effectiveness. The results over several seasons do indicate that in a protectant schedule dieldrin was the most effective material for plum curculio control followed by EPN, methoxychlor and parathion. It is interesting to note that no characteristic similarity in results was disclosed between materials of the same chemical groups.

Eradicant Program.—The effectiveness of materials tested in this manner has been determined during several seasons. While results vary seasonally due chiefly to the stages present at the time of treatment the relative performance of materials has been fairly constant. The results of tests in 1953 are cited as being rather typical. In this test a single spray was applied on June 11, considerable injury having occurred in the interval since shuck-split stage on May 25. At the time of treatment approximately 15 per cent of the eggs laid previously had hatched.

The results obtained are shown graphically in Fig. 2. The high degree of effectiveness of a single spray applied under these conditions is striking, all materials giving over 50 per cent control. Parathion was clearly the most effective reversing its position in the protectant schedule. Dieldrin was next followed by EPN and methoxychlor. The relative effectiveness of methoxychlor and EPN was comparable to their performance in the protectant program. The survivors of the dieldrin treatment were apparently larvae present at the time of treatment and these seem to have emerged slightly prematurely.

Further evidence relating to the ovicidal action of materials was gained by a test conducted earlier in which sprays were applied before any hatching had occurred. Samples of 100 or more injured fruits were collected several days after treatment and held in an insectary until completion of hatch. Examination was then made with the aid of a binocular microscope to determine the fate of eggs treated in this manner. The toxic effect of treatment was found to cause death at various stages of embryonic development. In some cases no development

Table 2.—The effect of spray treatment on hatching of plum curculio eggs in prunes.

Wettable Powder	Lbs. per 100 Gals.	Stage of Development—Per Cent: Egg: No. Embryonic Development Visible	Egg: Head Capsule Visible	Larvae: Dead at Point of Hatching	Larvae: Dead in Fruit	Larvae: Live	Per Cent Control
Parathion, 15%	2	24	63	9	0	4	96
EPN, 15%	1.2	12	70	0	6	12	88
Dieldrin, 50%	0.5	7	33	9	21	30	70
Methoxychlor, 50%	3.0	9	31	1	2	57	43
Untreated		4	1	2	0	93	—

was apparent by visual inspection while in others the embryo developed to the point that the head capsule was visible. Some embryos which succeeded in emerging from the chorion died before moving from the point of hatching. Others died in the larval stage after burrowing into the fruit and undergoing some growth. The results of these counts are given in table 2.

The degree of control shown here is not in complete agreement with the results shown in Fig. 2. This is not surprising considering that the stages present at the time of treatment were not identical. All materials showed some effectiveness as ovicides. Parathion was the most effective and the lethal effect occurred at an earlier stage of development. EPN was next in this respect. Chemically related materials showed some similarity with respect to total effectiveness and stage of development in which lethal effect was exerted.

Residual Effectiveness.—Insecticides might prove ineffective against the plum curculio for one or more of the following reasons: lack of toxicity, slowness of toxic action and insufficient residual persistence. As indicated earlier, field control tests do not disclose the influence of these factors individually. To provide such data insects were caged on treated foliage bearing known residue loads.

A single spray was applied to prune trees on June 15 in the conventional manner described in the foregoing section. At intervals after treatment branches bearing fruit were collected and placed under cylindrical screen cages in an insectary. Stems were placed in water to prevent wilting. Laboratory reared adults were introduced to cages through an opening provided in the top. Four replicates of 20 insects each were used for each test. The test insects were examined daily and records taken on morbidity and mortality. Insects were considered morbid when their activity was sufficiently affected to presumably render them incapable of feeding and ovipositon. The term morbid as used here is roughly synonymous with "knock down" although some beetles classified as morbid still clung to foliage. The distinction between morbid and dead is an arbitrary one as insects appearing as dead show high respiratory activity for a time.

Concurrent with cage tests, leaf samples were collected for residue determinations. Samples consisted of 100 disks, $\frac{7}{8}$ inch in diameter, punched from the center of mature leaves collected at random. Residue determinations on parathion and EPN were based on the method of Averell & Norris (1948). Methoxychlor was determined by the colorimetric method of Fairing & Warrington

Table 3.—Initial residue deposit on prune foliage in relation to quantity of toxicant in spray.

Wettable Powder	Lbs. per 100 Gals. Water	Concentration of Toxicant in Spray (p.p.m.)	Ratio to Parathion	Initial Deposit Micrograms/100 Sq. Cm.	Ratio to Parathion
Parathion, 15%	2.0	360	1	349	1
EPN, 25%	1.2	390	1.1:1	455	1.3:1
Dieldrin, 50%	0.5	300	0.8:1	270	0.8:1
Methoxychlor, 50%	3.0	1,800	5.0:1	2,712	7.8:1

(1950). Dieldrin residues were determined through the courtesy of Dr. Louis Lykken, Technical Service Dept., Shell Chemical Corp., Denver 1, Col. Data on initial deposit are given in table 3. In the case of parathion, EPN and dieldrin a rather precise relationship existed between concentration of toxicant in the spray mixture and initial deposit. Methoxychlor differed in giving a relatively heavier deposit.

Data on rate of weathering are presented graphically in Fig. 3. The configuration of the weathering curves for all materials was roughly similar showing no characteristic similarity between chemically related materials. The rate of weathering was highest for parathion, only 1 per cent of the initial deposit remaining after 14 days. Methoxychlor weathered most slowly with approximately 20 per cent present after 14 days. Dieldrin and EPN were quite similar with respect to rate of weathering being intermediate to the other two.

The insecticidal effectiveness of residues is presented graphically in Fig. 4. In all cases the break in effectiveness came between 4 and 7 days, the break being sharpest in the case of parathion, and least pronounced for dieldrin with the other two intermediate. With the exception of parathion differences in residual effectiveness were not striking. From the mortality curves and residue data the minimum effective residue can be interpolated. While such a calculation lacks precision it serves to indicate broadly the relative toxicity of the materials. This relationship is given below:

	Minimum Effective Residue Mg./100 Sq. Cm.	*Ratio to Parathion*
Parathion	34	1
EPN	68	2.0:1
Dieldrin	71	2.1:1
Methoxychlor	865	25.4:1

Parathion is clearly the most toxic of the materials with EPN and dieldrin comparable and methoxychlor strikingly less toxic.

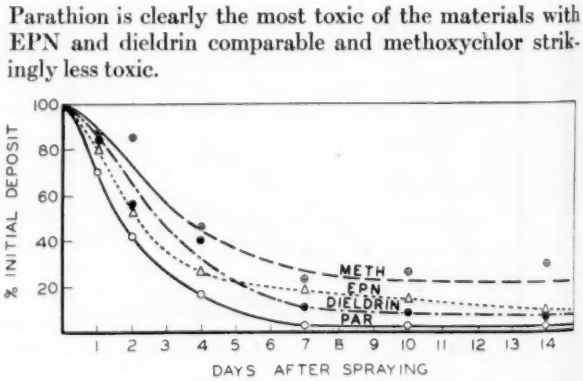

Fig. 3.—The rate of weathering of spray residues on prune foliage following single application on June 15, 1953.

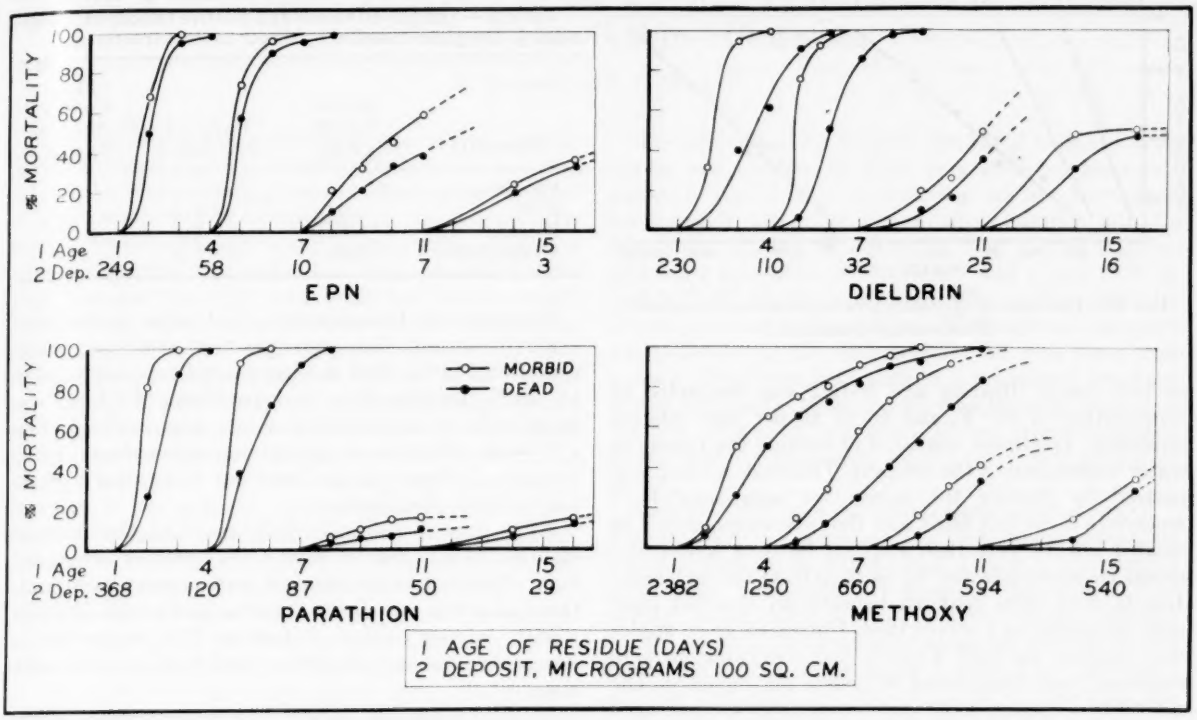

FIG. 4.—The insecticidal effectiveness of residues as determined by cage tests.

In addition to relative toxicity data the curves disclosed some similarity of toxic action between related materials. The thiophosphates were characterized by rapid morbidity and death with EPN showing less lag between the two curves. Dieldrin induced rapid knockdown with considerable lag before death. This point is not of practical significance as there was no evidence of recovery. Methoxychlor showed slow speed of action even by the initial residue deposit. In attempting to relate these findings to performance under field conditions two points seem significant, namely, the short residual effectiveness of parathion and the low toxicity and speed of action of methoxychlor. The residual effectiveness of EPN and dieldrin seemed comparable although dieldrin proved superior under field conditions (Table 1). More precise evaluation of materials by this method could perhaps be made if cage testing was concentrated at the breaking point. One difficulty is that residues which have weathered below the minimum effective dosage act more slowly requiring an extended period of observation and the prune foliage wilts before the test is finally concluded. This problem is not satisfactorily overcome by caging insects on the trees as other variables are introduced.

In addition, the method has another serious limitation in that records of injury occurring before death can not be readily obtained. This is due in part to the behavior of the test insects in response to the unnatural test conditions. As a result of crowding, insects at times feed in punctures produced by other insects thus avoiding the ingestion of surface residue. In other cases the insects feed on foliage, a situation uncommon to natural conditions.

The fact that in these tests all materials remained effective for less than 10 days seems at variance with the high degree of control obtained under field conditions by sprays applied at this interval. Several factors may account for this seemingly contradictory evidence. First, the test insects were reared in the laboratory and these have been found to be less susceptible than overwintering beetles. More valid comparisons could perhaps be made between these data and field control of second brood adults although these do not occur locally. Second, field results reported here were based on recovery of mature larvae which survived treatment rather than to injury by feeding and oviposition. The experimental method generally followed in tests on apples and peaches takes account of such injury thus subjecting materials to a more rigorous test.

Despite the limitations mentioned the method provides useful supplementary data relative to toxicity, speed of action, and residual effectiveness.

DIRECT CONTACT TOXICITY.—Investigations under this heading were concerned with the toxicity of materials when applied directly to test insects. This method of treatment offers several distinct advantages. It is a more precise method of exposure than residue cage tests where toxicity might be due to ingestion of spray residue as well as to contact with treated surfaces. In addition, tests can be conducted without seasonal limitations imposed on field studies provided laboratory cultures of test insects are maintained. In addition, the conduct of such tests requires relatively little time. The important consideration is whether such data have a practical bearing on results obtained under conditions of actual usage.

Laboratory reared adults, 2 to 4 weeks old, were used

FIG. 5.—The toxicity of insecticides to plum curculio adults by direct contact treatment.

as test insects. Rearing and testing was conducted at temperature of 80° F. and 60 to 80 per cent relative humidity. Treatment consisted of wetting the insects in water suspensions of the toxicant. Thorough wetting was insured by shaking the insects for approximately 5 seconds in a vial half filled with the toxic suspension. The treated insects were then emptied on to a screen and excess moisture removed by blotting from the underside. Insects were then confined beneath an inverted glass dish supported on a screen base. Untreated green apples were supplied for food. Tests at each concentration were replicated four times using 20 insects per replicate. The mortality occurring in the various replicates was averaged to determine the rate for each concentration.

The lines plotted from the toxicological data are shown in Fig. 5. The LD-50 values derived from the curves and the relative toxicity index using parathion as a standard are given in table 4. The order of toxicity is similar to that established for residual effectiveness. There are, however, striking differences in effectiveness of individual materials by the two methods, dieldrin and methoxychlor being less toxic by direct contact. The relationship between the thiophosphates was that expected from residual effectiveness, namely that parathion was approximately twice as toxic as EPN and the LD-50 values of both were well below the field concentration.

In the light of evidence pertaining to methoxychlor it is difficult to explain its effectiveness under field conditions (Table 1). By direct contact the LD-50 value exceeds the concentration employed in the field. This fact suggests that lethal effect does not result from external exposure. It is possible that the toxicant acts as a stomach poison when ingested in feeding or imbibing moisture. The latter factor was found to play an important role in the effectiveness of lead arsenate against this species. (Smith 1954). It is possible that the material acts partially as a repellent.

From these results it appears that direct contact toxicity is not a reliable index to the effectiveness of chlorinated hydrocarbons. In the case of the thiophosphates a rather close relationship existed between residual and contact toxicity. This is suggestive of different modes of entry by the two chemical groups. The absence of specific evidence along this line emphasizes the need of further studies on how lethal dosages are acquired.

Table 4.—The LD-50 values and relative toxicity of insecticides to the plum curculio by direct contact treatment.

MATERIAL	LD-50 VALUE (p.p.m.)	RATIO TO PARATHION	FIELD CONCENTRATION (p.p.m.)
Parathion	14	1	360
EPN	32	2.3:1	390
Dieldrin	104	7.4:1	300
Methoxychlor	4000	285.7:1	1800

SUMMARY AND CONCLUSION.—Tests were conducted to evaluate four materials under practical and experimental conditions. In the field dieldrin proved superior followed by EPN, methoxychlor and parathion. Dieldrin was found to have quick speed of action, long residual action and some effectiveness against immature stages. Effectiveness by direct contact was not in keeping with its high residual effectiveness.

EPN showed residual effectiveness of the order of dieldrin. In addition, it gave quick speed of action and high effectiveness by residual and contact treatment. Ovicidal action was slightly superior and larvicidal action slightly inferior to that of dieldrin. The reasons for its secondary position to dieldrin in field tests are not readily apparent.

Methoxychlor was outstanding in its heavy residual deposit and persistence but these factors were partially offset by low toxicity and speed of action. Effectiveness against immature stages was least of all. By contact action it was strikingly ineffective. The control obtained under field conditions hardly seems in keeping with these findings.

Parathion was weak in only one respect, short residual effectiveness. It showed highest toxicity by residual and contact treatment and was also most effective against immature stages. Despite these features it accounted for poorest control under conditions of actual usage indicating the importance of residual action in field control.

In assessing these materials some of the more obvious factors influencing effectiveness have been investigated. The considerable evidence gained does not permit valid predictions regarding effectiveness under field conditions. This is perhaps due to the influence of other factors such as temperature, humidity and rainfall which are subject to seasonal fluctuations. The evaluation of new materials by the criteria used here would likely establish their limitations without the prolonged field testing generally required.

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Low Temperature Tolerance of the European Corn Borer in Relation to Winter Survival in Minnesota[1]

D. Barnes[2] and A. C. Hodson[3]

Since the European corn borer has moved rapidly northward from population centers to the south and east into all the corn-producing areas of Minnesota, the effect of winter temperatures in that state on the ability of the borer to continue to survive and to maintain a population level of economic importance has been questioned. One may postulate that the corn borer has become established in the newly invaded areas either because the hibernating larvae are well protected from severe cold by insulation, or because they are sufficiently cold-hardy to withstand the low temperatures of Minnesota winters even in unprotected situations. It has been established by field surveys that there is usually a good survival over winter, even above the snow, in standing corn stalks. However, in the spring of 1948 a high percentage of the borers in the stalks were dead. Thus, we are certain that under some conditions a large proportion of the larvae are unable to overwinter successfully in Minnesota.

Insects may be classified into four general categories on the basis of their tolerance to cold. These are: 1) insects which are able to survive freezing and are killed only by long exposure to low temperatures or by one or more sudden changes in temperature; 2) insects that can remain dormant in an undercooled state but do not survive freezing at temperatures below the undercooling point; 3) insects which exhibit very little undercooling and are killed by temperatures near the true freezing point; 4) non-hardy, non-hibernating insects killed by temperatures above freezing.

The purpose of this study is to determine which of the above categories characterizes the corn borer and consequently the nature of the cold hardiness of this species; and further, to establish the time during the dormant period when natural mortality is most likely to occur. Most of the emphasis has been placed on a study of the importance of the "intensity factor of cold" as indicated by determining the undercooling point and freezing of larvae collected periodically from the field, and of larvae exposed to experimental conditions in the laboratory. Dittman (1943), states that the undercooling of living insects behaves in the same manner as water in a finely divided state. Water particles in this condition, in the absence of ice crystals or other solid particles that may act as nuclei for crystallization in the absence of shock or movement, may be undercooled to a considerable degree.

Methods and Material—Undercooling point determinations for the larvae were conducted at refrigeration temperatures of −32° C. The rubber thermo-couple holder and refrigeration equipment used for all the undercooling point determinations are described by Salt (1936). In order to observe recovery following treatment, the undercooled larvae were carefully removed from the thermo-couple holder and placed on saturated blotting paper in a petri dish and held at 20° C. All other temperature studies were conducted in controlled temperature cabinets in which there was a temperature variation of ±0.5° C.

The larvae used for study in the fall of 1948 and in the spring and autumn of 1949 were collected from corn stalks in the field. Undercooling studies were made immediately after the larvae were brought into the laboratory. During the autumn of 1948 a stock of larvae of known generation was collected and stored at 5° C. and 80% R. H. These were used for study during the winter. Another group of larvae, likewise used for experimentation during the winter of 1948–49, were taken from a stock pile of corn kept outside in a screened insectary. It was not possible to be sure whether these larvae were of the first or second generation. Unless otherwise stated, larvae of this supply were used in the winter laboratory study.

Seasonal Studies on Field Collected Larvae.—*Fall aspect.*—On September 11, 1948 second generation fourth instar larvae were collected from a late-planted sweet corn field near Montgomery, Minnesota. The average undercooling point of 14 specimens was found to be 10.7 ± 1.4° C. Determinations made in 1949 on second generation fourth instar larvae showed good agreement with the 1948 data. In both cases, after undercooling, the larvae were found to be frozen hard, and none survived the freezing process. In 1948 the average undercooling point of third instar larvae was 8.6° C., and in 1949 it was found to be 7.8° C. Like the fourth instar larvae, the third instar larvae did not survive temperatures below their undercooling points.

Beginning July 31 and at approximately weekly intervals thereafter, undercooling points were determined for fifth instar larvae, table 1. From July 31 until September 27, the larvae treated were most certainly of the first generation; however, following that period, the larval generations could not be separated with certainty.

Table 1.—Undercooling points of fifth instar corn borer larvae taken at intervals throughout the fall of 1949.

Date	Number of Larvae Tested	Average Undercooling Point	Per Cent Water	Survivals
July 31	10	−14.6 ±0.35° C.	63.5	None
Aug. 17	10	−11.5 ±0.11	53.0	None
Sept. 6	7	−16.1 ±0.87	58.6	None
Sept. 13	10	−16.2 ±1.65	55.4	None
Oct. 8	7	−18.9 ±0.89	54.8	All dead[a]
Oct. 23	7	−22.0 ±0.96	52.7	1 dead[b]

[a] Two days after treatment.
[b] Following treatment. Remainder survived.

As shown in table 1, there was a gradual decrease in the undercooling point and in the water content of the larvae as the fall season advanced. Furthermore, the ability of the larvae to survive freezing exhibited a marked

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change late in the season. None of the larvae was able to survive freezing on October 8 when the undercooling point had reached an average of about −18.9° C. Fifteen days later, on October 23, when the undercooling point reached an average of about −22.0° C., all but one of the larvae tested survived freezing after being subjected to temperatures below the undercooling point. These survivors were placed at 20° C. in contact with saturated blotting paper and 6 weeks later all had spun cocoons.

From these data it may be concluded that the ability of the larvae to survive freezing becomes apparent only after the undercooling point reaches a range of from about −18° C. to −22° C. As will be shown in the discussion of the winter aspect of the problem, the undercooling point level becomes somewhat lower during the winter and the ability of the larvae to withstand freezing persists throughout the winter. Thus, the ability to survive freezing was considered to indicate that the borers had reached a state of maximum cold-hardiness.

It was established that younger larvae and early fifth instar larvae are killed when frozen. However, there was still the possibility that these non-hardy individuals could stand long exposure to moderately low temperatures above their undercooling point, such as might occur during the fall season. To explore this point, 5 lots of 10 fourth, and 5 lots of 10 non-cold-hardy fifth instar larvae were placed at 5° C. in contact with saturated blotting paper. The larvae were regarded as dead only if they did not respond to gentle probing after they had been held at room temperature for 24 hours. The results showed that 94% of the fourth instar larvae succumbed within 1 week, while about 18% of the fifth instar larvae died in the same period of time. A mortality of 26% for fifth instar larvae resulted at the end of 2 weeks. These data indicate that larvae collected early in the season show a low tolerance to moderately low temperatures well above the undercooling point. Since some larvae go into the winter either in instars younger than the fifth or in a non-cold-hardy condition in the fifth instar, considerable mortality may be expected whenever these stages are abundant in the late fall.

It was of interest to examine the records of the Minnesota State Entomologist's field survey data for 1947–48, in the light of these results on the fall cold tolerance of the borers. It was found that 71.2 % of the borers entered the winter season in 1947 in instars from one to four. In 1948 only about 30.4% entered the winter in similar stages of development. The significance of the difference in the age composition of the fall populations for these 2 years is apparent when it is compared with the mortality figures obtained from the spring survey records. The comparison is made in table 2. Survey personnel reported that at no time during the spring did they find living larvae in any stage other than the fifth instar. From table 2 it would appear that the high mortality recorded in the spring of 1948 is correlated with the very high proportion of first to fourth instar larvae present late in the fall of 1947. In addition, many of the fifth instar larvae may not have become cold-hardy soon enough.

The year 1947 was characterized by a late cool spring, cool summer, a short warm autumn, and a sudden onset of cold winter weather. 1948 was much closer to what might be termed a "normal" weather year characterized by an average spring, long growing season, and a long dry fall.

The temperatures of the air in the corn stalks and in the borer tunnels during the months of September, October, and November agreed fairly closely. One may conclude from this that the protection which dry corn stalks offer to corn borer larvae against sudden or extreme changes of air temperature is not likely to be of great importance during the winter.

Winter aspect.—During the winter and spring of 1948 and 1949, many larvae in the corn stalks were not only frozen hard but were completely surrounded by ice crystals, while other larvae, often in the same stalk, were soft and free from ice. On February 9, 1949, 10 hard larvae and 10 soft ones were brought into the laboratory and undercooled. The average undercooling points were -29.0 ± 0.35° C. for the hard larvae and -28.6 ± 1.15° C. for the soft ones. All larvae from both series survived this treatment, even though they were cooled again to their undercooling points after the rebound had occurred. It is believed that the soft larvae taken in the field were in the undercooled but unfrozen state and that the larvae frozen hard had been cooled below their undercooling points.

From January through March, the undercooling point level for larvae collected from corn still standing in the field averaged about −29° C.

Spring aspect.—Beginning March 5, 1949, undercooling point determinations were made on overwintering larvae collected from corn fields around the Twin Cities to determine, over a 9-week period, what effect the seasonal change of spring temperatures would have on the undercooling point and survival after freezing.

As shown in figure 1, little change occurred until April 1. During April, there was a gradual but very slow rise in the undercooling points and on May 7, well past the danger of any very harmful frost, the average undercooling point was still at a low level (−20.4° C.). The larvae survived this treatment in every case, thus indicating a state of maximum cold-hardiness.

As the spring temperatures tend to moderate somewhat earlier in the southern part of the state, it was considered possible that the undercooling point level for the overwintering corn borer larvae from this area might begin to show response, by a gradual rise, earlier than that for larvae in corn fields in the more central part of the state. On April 23 a group of fifth instar larvae were obtained from the town of Blue Earth, Minnesota. Their undercooling points averaged −26.3° C., approximately 1 degree lower than for material collected near the Twin

Table 2.—Age composition of fall corn borer larval populations for 1947 and 1948 compared with spring records of mortality.

	Autumn Composition		Spring Mortality	
	Instars I–IV	Instar V	1948	1949
1947	71.2%[a]	24.1%	80.7%[b]	—
1948	30.4%[a]	64.3%	—	24.2%[c]

[a] 60 counties.
[b] 36 counties.
[c] 22 counties.

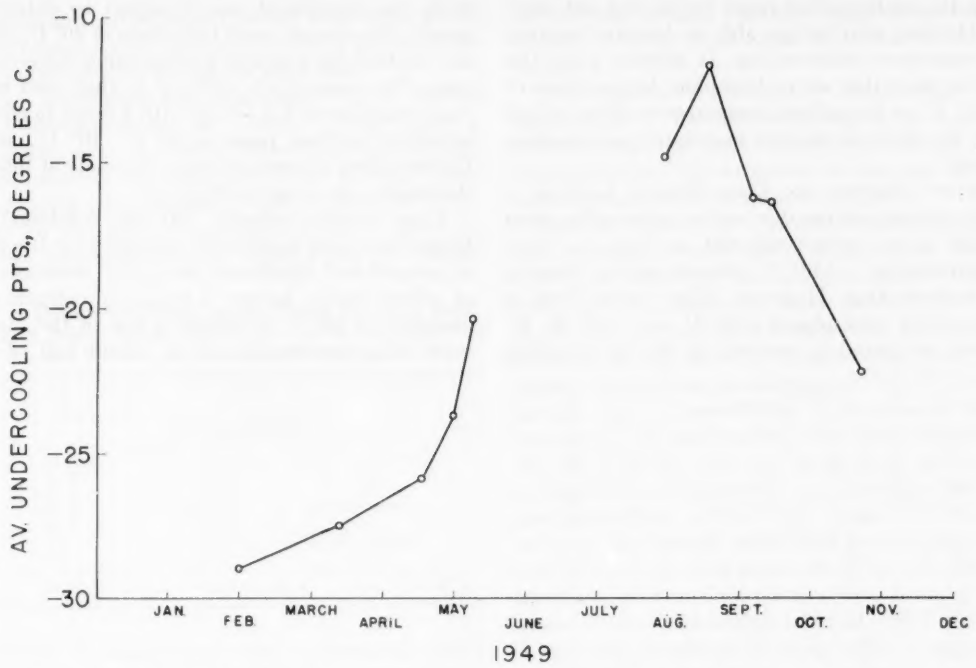

FIG. 1.—Graphical presentation of the observed undercooling points for fifth instar larvae during the months of March and April and the first week of May compared with air temperature averages.

Cities on the same date. These larvae survived the undercooling and subsequent freezing. In this case, the borers from the southern part of the state responded to warmer spring conditions in about the same way as borers collected in the vicinity of the Twin Cities.

The seasonal trend of the observed undercooling point levels for fifth instar larvae is summarized graphically in figure 2. No determinations were made between May 7 and July 31, 1949. In the spring there was a gradual rise in the undercooling points, but the larvae maintained their ability to survive freezing through May 7, 1949. Beginning with the first of August there was a gradual

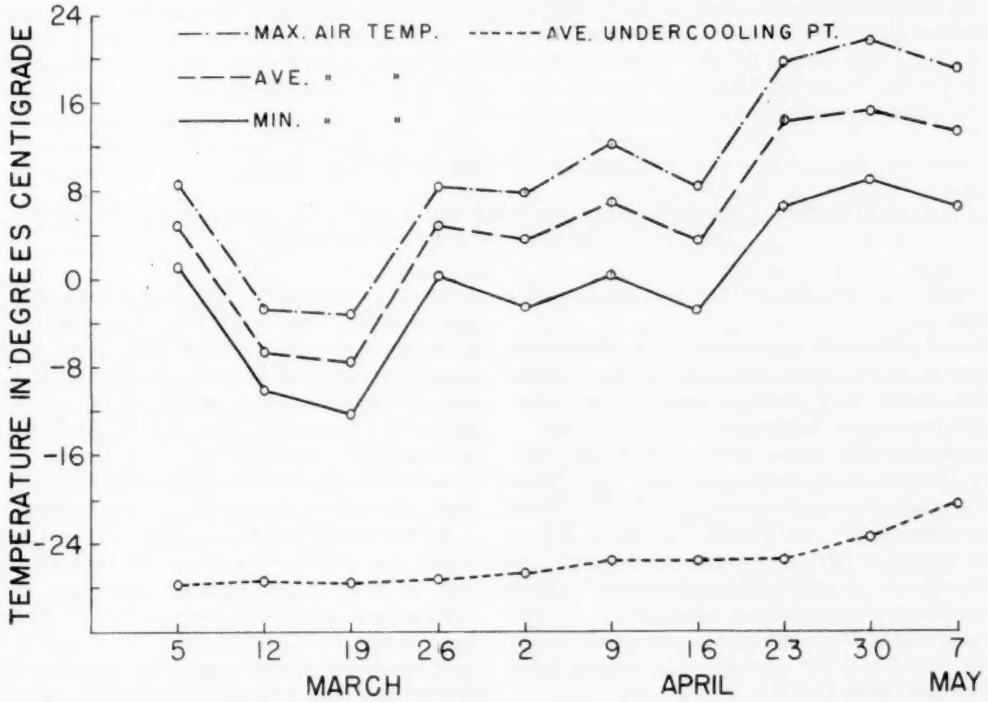

FIG. 2.—Seasonal variation of the average undercooling point levels of fifth instar corn borer larvae.

decrease in the undercooling point levels, but not until after mid-October were larvae able to tolerate freezing following maximum undercooling. It appears from the work done to date that an undercooling temperature of about −20° C. is the critical temperature above which larvae are not able to survive maximum undercooling and freezing

LABORATORY STUDIES ON CORN BORER LARVAE.—*Effect of temperature and moisture on the undercooling point levels.*—Fifth instar larvae collected on Sept. 2, 1949, which undercooled at −15.6° C., did not survive freezing following undercooling. However, other larvae from a similar lot, which were placed at 5° C. and 75% R. H. for 1 month, exhibited a lowering of the undercooling point to −20.7° C., and did survive freezing following undercooling.

A number of additional experiments were undertaken to determine to what extent conditions of temperature and moisture would bring about a change in the undercooling point level of the European corn borer larvae.

First generation fifth instar larvae, which had been collected at Waseca on September 22, 1948, were found to have an average undercooling point of −19.5° C. These larvae were stored at 5° C. and 75% R. H., and by February 8, 1949, 20 weeks later, the undercooling point had dropped to −27.4° C. A series of these larvae were placed in contact with saturated blotting paper at 20° C. One week later a group of these larvae, not previously undercooled, was tested and the average undercooling point was found to have risen 11.1° C.

Since the undercooling point rose so rapidly within a week, an experiment was designed to determine how rapidly this change could take place at 20° C., and to find out whether the response was the same at lower temperatures. The same stock material as that used in the previous experiment was set up with a series in contact with saturated blotting paper at 5° C., 10° C., and 20° C. Undercooling determinations were made at intervals and the results are given in figure 3.

These results indicate that the combined effect of temperature and moisture is necessary for the production of a rapid and significant rise in the undercooling point of winter-hardy larvae. Larvae in contact with free moisture at 20° C. exhibited a rise in the undercooling point of approximately 11° C., about half of which appeared to occur in the first 2 or 3 days after they were placed under these conditions. The undercooling points of larvae in contact with free water at 10° C. did not rise either as rapidly or as much. At the end of a 17-day interval the undercooling points of the second group of larvae had risen only about 8.5° C. At 5° C. the undercooling point rose about 4° C. in the first 2 days and remained at approximately this level for the following 23 days, after which the experiment was terminated.

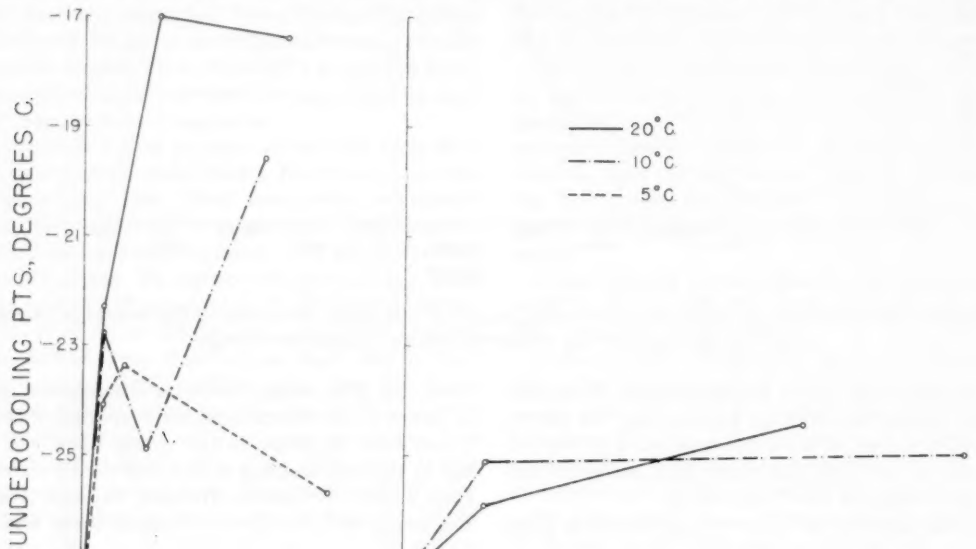

FIG. 3.—The effect of temperature and moisture in bringing about a rise in the average undercooling point level of cold-hardy European corn borer larvae.

In contrast to the winter-hardy larvae, these larvae in which the undercooling point had been raised were not able to survive freezing after maximum undercooling. Lozina-Lozinskii (1935) reports that if European corn borer larvae were held at 20° C. or more for some time, they would freeze more easily. He also states that larvae treated this way were killed by undercooling at −5° C. or −7° C. Dittman (1943) found that undercooling

temperatures and subsequent freezing were fatal to all the species he had under observation except larvae of the cold-hardy, hibernating European corn borer.

A further experiment was conducted with a limited number of larvae collected on March 7, 1949, from a sweet corn field near the Twin Cities. These larvae had an average undercooling point of −27.2° C. They were placed in contact with saturated blotting paper at 5° C., 10° C. and 20° C., and the undercooling points given in table 3 were determined 4 days later. From the results given in table 3, it would appear that in general the effect of temperature and moisture in bringing about a rise in the undercooling point level of cold-hardy fifth instar larvae collected from the field very early in the spring is approximately the same as that of larvae stored under laboratory conditions.

Table 3.—The effect on the undercooling points of field-collected fifth instar larvae following a 4-day exposure at 20° C., 10° C. and 5° C. in the presence of free water.

Larvae Number	20° C.	10° C.	5° C.
	Undercooling Points		
1	−24.0° C.	−24.6° C.	−26.4° C.
2	−16.5	−25.0	−26.0
3	−18.3	−26.3	−27.0
4	−22.0	−27.0	−25.8
5	−21.3	−26.7	−26.6
6	−17.3	−24.2	−25.6
7	−20.1	−26.5	−26.1
Average	−19.9±1.48° C.	−25.6±0.36° C.	−26.2±0.19° C.

In the study thus far, it seemed possible that the rise in the undercooling point at the higher temperatures, i.e., 20° C. and 10° C., might be affected by the presence of free mosture. In an effort to separate the effects of temperature and moisture at these temperatures, another series of larvae was placed at 5° C., 10° C. and 20° C. in dry vials over a saturated sodium chloride solution—a relative humidity of about 75%. Undercooling point determinations were made at varying time intervals and the results are given graphically in figure 3.

From an examination of the data in figure 3, it is apparent that moderately high temperatures (10° C. and 20° C.), in the absence of free moisture, did not cause the undercooling point level to rise either as rapidly or as much as when free moisture was present. Further, larvae held at 5° C. and free from contact moisture exhibited no change in the undercooling point level. However, in the presence of free moisture at 5° C. the undercooling point level rose about 4.5 degrees in the first 4 days and then fell gradually to about −26.5° C. These observations indicate that it is the effect of temperature and moisture acting together which brings about the rapid and marked rise of the undercooling point level of cold-hardy fifth instar corn borer larvae.

In the light of these observations on the effect of contact moisture, it is important to know whether a similar condition exists in the corn plants as they stand in the field. The hygroscopic coefficient and moisture determinations that have been made indicate that there is sufficient moisture in apparently dry corn stalks early in the spring to provide contact moisture for the borers. However, spring mortality from freezing is unlikely because it is not until early in May, after the danger of severe frost is past, that the temperature becomes high enough to bring about a noticeable rise in the undercooling point.

The opposite view is held by Monchadski (1935), who states that in Russia the spring is an important and critical period for corn borer larvae because it is during this period that the recurrence of cold and drought could cause a great reduction in borer numbers.

The dependence of the undercooling point level on environmental conditions of temperature and moisture is further shown by the following observations. Larvae from the first generation stock held at 5° C. and 75% R. H. were placed in contact with saturated blotting paper at 20° C. and the undercooling point was subsequently raised from an average of −27.5° C. to an average of −19.1° C. on March 8. A group of these larvae, not previously undercooled, were then returned to 5° C. and 75% R. H., and on April 19, 6 weeks later, the average undercooling point was found to have been lowered again from −19.1° C. to −25.1° C. When another series of the larvae, which had been returned to 5° C. and 75% R. H., was again tested on July 26, 20 weeks later, the undercooling point averaged about −25.9° C. Those larvae which undercooled at −25.1° C. and −25.9° C. were able to survive freezing, while those undercooling at −19.1° C. were not.

The "quantity effect" of low temperatures on cold-hardy fifth instar larvae.—The ability of winter-hardy larvae to survive rapid changes in temperature as well as temperatures as low as the maximum undercooling point has been noticed in previous experiments. To determine how long larvae in the cold-hardy state can survive low temperatures, in other words the "quantity factor" of low temperatures, the following experiment was conducted. On February 20, 1949, 20 winter-hardy larvae, exhibiting an average undercooling point of −27.6° C., were surface-dried with a blast of air and placed in dry vials at −32° C. for 18 and 22 days. From these larvae, 10 males and 10 females developed, mated, and produced viable eggs. Still longer exposures might have produced different results (Salt 1950).

To study the effect of contact moisture on the survival of cold-hardy larvae at low temperatures, one group of 15 larvae in contact with free moisture and another in a dry condition were placed at −32° C. On examination 1 week later, all the larvae in contact with free moisture were dead, while those in the dry state were alive; when placed in contact with saturated blotting paper at 20° C. the latter completed their development. In a similar test in which two lots of 25 larvae were used, it was found also that all the wet larvae were dead within the first week, while of the dry larvae three were still alive at the end of 98 days. Two of these died while in the larval stage, while the remaining one pupated on August 17, and emerged on August 27. Observations and experiments made by Hodson (1937), Beal (1933), Miller (1931), and Salt (1936), showed that for the respective insects under study contact water may be responsible for the increase of mortality at low temperatures. In most of these cases the insects were subjected to a sudden exposure of a very low temperature.

Effect of repeated undercooling of cold-hardy larvae.—Repeated undercooling point determinations were made

on winter-hardy larvae by undercooling them three times. Following each treatment the specimen was allowed to thaw and then was surface-dried with a blast of air and undercooled again, all within 10 to 15 minutes. Although the undercooling points did not change appreciably, varying between 1° to 4° C. with this treatment, none of the larvae survived.

During the winter months the temperature may rise high enough to bring the larvae out of the undercooled condition, but the danger of mortality from rapid and repeated freezing and thawing during the winter is unlikely to occur commonly under Minnesota conditions. In early spring unseasonable periods of warm weather might bring larvae out of the undercooled state, and under these conditions a sudden drop in temperature might be fatal to dormant larvae. However, at this time of year temperature fluctuations great enough to be comparable with the repeated laboratory undercoolings do not occur very often.

SUMMARY.—Studies were made in Minnesota on European corn borer larvae to determine a) the low temperature tolerance of the larvae with respect to their ability to survive both the "intensity" and "quantity" factors of cold, and b) the effect of temperature and moisture in producing either a rise or a depression of the undercooling point level.

In the fall of 1949, fourth and early fifth instar larvae collected from the field were not cold resistant. From this it is concluded that there is a correlation between age composition of the fall population and ability to overwinter successfully. This conclusion is confirmed by field survey data for 1947–48, which show that in the late fall of 1947 there was an abundance of larvae in stages younger than the mature fifth instar and that mortality for the following winter was high.

In mid-October corn borer larvae survived freezing following maximum undercooling only after the undercooling point reached a temperature of approximately −20° C.

During the winter the undercooling point was of little significance as an indicator of cold-hardiness, since cold-hardy larvae survived freezing for at least 3 weeks in the laboratory and for the entire winter in the field.

In the spring of 1949 the undercooling point level of dormant larvae did not rise above approximately −20° C., and the larvae maintained their ability to survive freezing long after the danger of exposure to critical low temperatures was past.

Experiments showed that the combined effect of moderately high temperature and contact moisture is necessary to bring about a rapid rise in the undercooling point of cold-hardy larvae.

Conditions preceding and during the fall season are considered a more important cause of high mortality among hibernating corn borer larvae than winter and spring conditions.

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Control of the Green Peach Aphid on Burley Tobacco[1]

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The green peach aphid, *Myzus persicae* (Sulz.), has been recognized as an important, but sporadic pest of burley tobacco in Tennessee and Kentucky for the past 7 or 8 years. Prior to this, in 1946, it was noted as an apparently new and serious pest in the Georgia-Florida shade-grown tobacco belt and in the flue-cured tobacco belt of the Carolinas and Virginia.

As a result of the economic losses caused by this aphid, numerous research workers have conducted chemical control investigations. Creighton & Gresham (1947), Dominick (1949), Chamberlin (1949), and Kulash (1949) demonstrated the effectiveness of parathion in controlling the green peach aphid. Dominick, as well as Kulash, showed that TEPP resulted in a quick, high initial knockdown of this insect. However, as a general rule the materials that have been shown to control the green peach aphid are also recognized as being highly toxic to warm-blooded animals. For this reason, additional information about materials less toxic to man but effective against this pest would be of value. Also, as plant coverage is directly related to aphid control, data on the effects of systemic insecticides on aphid populations would be of interest, especially in the tobacco plant bed where the plants are growing close together.

For the past 2 years aphid infestations of tobacco have been spotty in central Kentucky. During this time, infestations were largely restricted to low spots in the

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director. Accepted for publication December 5, 1955.

field and areas receiving shade such as field margins adjacent to woods or buildings.

METHODS.—*Laboratory tests.*—For laboratory insecticide screening tests, leaves heavily infested with aphids were collected in the field and placed on tobacco in the greenhouse. As the infested leaves wilted the aphids readily moved to the greenhouse plants. In most tests, clay pots, 6 inches in outside diameter, contained a single burley tobacco plant of the variety Kentucky 58. These plants had an individual spread of approximately 6 inches and were uniform in size. In addition, tobacco germination flats, measuring 20″ by 26″ by 6″ and containing approximately 200 plants each, were used in a test with a systemic insecticide. The plants in these flats averaged about 5 inches in height in normal growth position. The effectiveness of the treatments in this test was determined by counting the living aphids on 5 plants selected at random from each flat 1 and 3 days following treatment.

The tests were conducted from 10 to 14 days after the initial artificial infestation. At this time each plant was infested with approximately 300 to 400 aphids which were nearly all of the wingless form. Thirteen insecticides were tested for their effectiveness against this insect. All of the treatments were applied as foliar sprays with a 2-gallon knapsack sprayer equipped with a single *TeeJet* 1.97 cone nozzle and operated at a pressure of 30 to 40 p.s.i. Treatments applied to tobacco in the clay pots were replicated four times; each replicate consisting of a single plant which was sprayed for a 3-second interval. The test involving tobacco in the flats had a single flat per treatment with 10 seconds of spraying time allotted to each flat. All of the materials except the systemics were 25% (by weight) emulsifiable concentrates, and each of these with the exception of 40% nicotine sulphate[2] was formulated from technical material dissolved in xylene. Five per cent (by weight) Triton X-171[3] was used in all cases as the emulsifying agent. A treatment consisting of the solvent and emulsifier was included for comparative purposes. A preliminary test indicated that solvent and emulsifier used at 50 p.p.m. in water had no appreciable effect on the aphids. Systemic insecticides were commercial or experimental formulations prepared by the respective manufacturers.

Aphid knockdown was observed 3 to 5 hours after treatment. To facilitate the knockdown counts, a filter paper 15 centimeters in diameter and fitted with a central hole large enough to accommodate the stem of the plant was placed over the soil in each pot. Counts of aphids on the plants 2 days after treatment were recorded. To determine the possible effects of the treatments on reinfestation or build-up of the aphid population, counts of living aphids on the plants were also taken 7 days following treatment. On plants where aphids were numerous and individual counts were difficult, population estimates were made after counting a portion of the individuals. Constant temperature and humidity were not maintained during the tests, but extreme variations of both were prevented.

Field test.—A field test was conducted on burley tobacco of the variety Kentucky 16 approximately 2 weeks prior to harvest. The experiment was in the form of a randomized complete block, replicated three times.

Table 1.—Preliminary test for control of the green peach aphid on burley tobacco in the greenhouse.

MATERIAL[a]	APHID KNOCKDOWN AFTER 5 HOURS[b]	APHIDS ALIVE AFTER 2 DAYS[b]	PER CENT REDUCTION OVER CHECK
Parathion	179	14	99
Chlorthion	211	64	96
Malathion	55	450[c]	70
Lindane	7	1500[c]	0
Endrin	3	700[c]	53
Nicotine sulphate	1	1200[c]	20
Check-solvent & emulsifier	1	1500[c]	—
Dipterex	0	1950[c]	40
DDT	12	2450[c]	25
Check-solvent & emulsifier	0	3250[c]	

[a] All materials including the control were used at 50 p.p.m. in water.
[b] Total from 4 replicates of 1 plant each.
[c] Population estimated.

Each plot consisted of 2 rows of tobacco 42 inches apart and 60 feet long. To minimize drift, six untreated rows were left between each treatment. Alleyways 16 feet in width separated each replicate. At the time of treatment the tobacco had been topped, was approximately 5 feet tall, had from 16 to 20 marketable leaves, and 2 to 6 suckers 6 inches or longer.

The materials, all in dust form, were applied by hand with a rotary hand duster at the rate of 30 to 40 pounds per acre. The wind during application was 3 to 5 m.p.h., relative humidity 40% and temperature 82° F. No rain fell during the test period. To determine the effectiveness of the materials being tested, aphid counts were taken from two adjacent plants near the center of each row in each plot 48 hours following treatment. These counts were compared with the untreated check.

RESULTS AND DISCUSSION.—*Laboratory tests.*—Table 1 shows the results of a preliminary laboratory test utilizing eight compounds at a uniform dosage of 50 p.p.m. in water. The test was designed to compare the relative toxicities of selected insecticides as an aid in determining materials to be used in subsequent tests. Currently used materials for aphid control on tobacco such as parathion and malathion were included. In this test, parathion and Chlorthion[4] were the only materials resulting in a 95% reduction of aphids over the untreated check 2 days after treatment. They also showed the highest knockdown of aphids 5 hours after being applied.

Five materials at three levels each, as well as an untreated check, are compared in table 2. Again parathion and Chlorthion at three comparable dosages appeared nearly equal in toxicity, and after 2 days and 7 days resulted in the greatest reduction in aphids as compared with the other treatments. The percentages of reduction over the check were too high, even at the lowest dosage, to differentiate among the levels. Both of these insecticides gave high aphid knockdowns even at the lowest

[2] "Black Leaf 40" manufactured by Tobacco By-Products and Chemical Corporation, Richmond, Virginia.
[3] Manufactured by Rohm and Haas Company, Philadelphia, Pennsylvania.
[4] Manufactured by Chemagro Corporation, New York, N. Y. under license from Farbenfabriken Bayer A. F., Germany

Table 2.—Laboratory tests using three dosage levels of insecticides for control of the green peach aphid on burley tobacco.

Material	Concentration (P.P.M. in Water)	Aphid Knockdown After 5 Hours[a]	Aphids Alive After 2 Days[a]	Per Cent Reduction Over Check	Aphids Alive After 7 Days[a,b]	Per Cent Reduction Over Check
Parathion	12.5	777	41	99	375	92
	25	620	35	99	547	88
	50	916	10	100	338	92
Chlorthion	12.5	823	396	88	775	83
	25	939	22	99	450	90
	50	617	1	100	275	94
Malathion	50	483	426	87	1500	67
	100	781	433	86	1009	78
	200	808	36	99	290	94
Lindane	100	239	2000	37	5400	0
	200	303	707	78	2850	37
	400	903	62	98	438	90
Endrin	80	297	335	89	475	89
	160	475	217	93	290	94
	400	609	97	97	45	99
Check (solvent & emulsifier)	50	113	3180	—	4500	—

[a] Total from 4 replicates of 1 plant each.
[b] Low populations were counted, high populations were estimated.

concentration tested. Malathion resulted in good aphid knockdown, especially at the two higher concentrations; however, this insecticide resulted in aphid kills lower than parathion or Chlorthion although it was used at concentrations four times greater than that of the other phosphates. Lindane and endrin gave aphid knockdown in direct proportion to the concentration. Lindane gave a distinctly greater kill of aphids as the dosage was increased. However, neither lindane nor endrin, considering the dosages used, was as effective as the phosphate insecticides.

Thorough plant coverage with an effective insecticide is necessary if aphids are to be controlled; however, coverage on tobacco is difficult to obtain because of the growth characteristics of the plant. For this reason, systemic insecticides, which might result in good control if applied to only a portion of the leaf, would be of value. A screening test, shown in table 3, consisting of five materials thought to be systemic or partially systemic in action was conducted. Am. Cyanamid 12008 and 3911, demeton, and Shell OS-2046 were highly effective at a concentration of 50 p.p.m. Bayer compound 17147 was

Table 3.—Results of systemic insecticide tests for control of the green peach aphid on burley tobacco in the laboratory.

Material[a]	Aphid Knockdown After 5 Hours[b]	Aphids Alive After 2 Days[b]	Per Cent Reduction Over Check	Aphids Alive After 7 Days[b]	Per Cent Reduction Over Check
Am. Cyanamid 12008	515	11[c]	99	186	94
Am. Cyanamid 3911	500	13[c]	99	285	91
Shell OS-2046	393	39[c]	99	765	76
Demeton	173	120	96	730	77
Bayer 17147	421	535	84	806	75
Check	0	3250[d]	—	3200[d]	—

[a] All insecticides and the control used at 50 p.p.m. in water.
[b] Total of 4 replicates of 1 plant each.
[c] Represent winged forms.
[d] Population estimated.

Table 4.—Laboratory tests with three dosage levels of Shell OS-2046 sprayed on burley tobacco in germination flats to control the green peach aphid.

Material	Concentration (P.P.M. in Water)	Number Aphids Alive After 1 Day	Per Cent Reduction Over Check	Number Aphids Alive After 3 Days	Per Cent Reduction Over Check
Shell OS-2046	50	24	99	121	95
	25	48	98	226	91
	12.5	368	83	445	82
Check	—	2150[a]	—	2425[a]	—

[a] Population estimated.

also effective at this dosage but to a lesser degree than the above-mentioned materials.

Although no counts were made immediately following treatment, Shell OS-2046 and Am. Cyanamid 12008 appeared to result in the quickest initial kill. Approximately 35 to 40 minutes following treatment very few live aphids could be found on these treatments.

Observations made on tobacco germination flats treated at three dosage levels with Shell OS-2046 on an unreplicated basis are shown in table 4. The reduction of live aphids over the check at 1 and 3 days at concentrations of 25 and 50 p.p.m. was above 90 per cent and thought to be excellent, especially considering the density and rank foliage of the young plants in the flats and the relatively high aphid infestation.

Field Tests.—The non-systemic materials used in the field tests, as shown in table 5, were selected on the basis of their performance in the laboratory tests, as well as their current use in the control of aphids and other tobacco pests. For example, the effect of endrin on aphids would be of interest as this insecticide is widely used for the control of hornworms, grasshoppers, and budworms on tobacco.

The aphids were located largely on the under, basal portions of the leaves and on the new sucker growth. In general, the aphid population was greater in replicate 1, which bordered the field, than in replicates 2 and 3 which were located progressively deeper in the field.

All of the materials in the test resulted in a significant reduction of aphids over the untreated check. One per cent parathion and 1 per cent Chlorthion appeared equal, or nearly equal, in toxicity and considerably more toxic than 4% malathion. Lindane and endrin were comparable in effectiveness but not as toxic to the aphids as the above materials. However, materials such as endrin and lindane, unless effective for a long period of time, would be un-

Table 5.—Green peach aphids present in field plots of burley tobacco 48 hours after receiving one application of insecticide.

Insecticide Dusts and Concentration	Pounds Active Ingredient per Acre	Aphids per 4 Plants				Per Cent Reduction Over Check
		Rep. 1	Rep. 2	Rep. 3	Mean	
Parathion 1%	0.4	151	127	144	141	97.9
Chlorthion 1%	0.3	155	250	207	204	97
Chlorthion 2%	0.6	190	248	125	188	97.2
Chlorthion 3%	1.2	2	6	17	8	99.9
Malathion 4%	1.7	1,560	470	560	863	87.2
Lindane 1%	0.3	2,340	2100	2180	2207	67.2
Endrin 1%	0.4	1,790	1640	1220	1550	77.0
Endrin 2%	0.6	2,880	1820	1090	1930	71.3
Check		10,730	5550	3920	6733	

L.S.D. at 5% level = 1972.

satisfactory because of the high biotic potential of this insect. For a material characterized by short residual action, such as the insecticides currently recommended for aphid control, high initial kill is necessary if rapid build-up is to be prevented.

SUMMARY.—Preliminary laboratory and field tests, utilizing eight non-systemic and five systemic materials, were conducted against the green peach aphid, *Myzus persicae*, on burley tobacco. In the laboratory tests, parathion and Chlorthion appeared highly effective (over 95% reduction over check) and equal, or nearly equal, in toxicity; and about four times as effective as malathion. In these tests, lindane and endrin were comparable in toxicity but less effective than the above mentioned materials.

In a laboratory test utilizing systemic insecticides at a concentration of 50 p.p.m., Am. Cyanamid 12008 and 3911, Shell OS-2046, and demeton resulted in 95 per cent or better reduction of aphids as compared with the check.

Dust formulations of parathion, Chlorthion, malathion, lindane, and endrin were used in a replicated field experiment. As in the laboratory tests, parathion and Chlorthion were highly effective, and equal or nearly equal in toxicity. The above materials were also more effective at 0.3 and 0.4 pound toxicant per acre than malathion at 1.7 pounds toxicant per acre. Lindane and endrin appeared to be nearly equal in toxicity, but these insecticides resulted in less than 80 per cent reduction of aphids.

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Control of House Flies in Dairy Barns with Special Reference to Diazinon[1]

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Extensive tests of phosphate insecticides for control of the house fly in dairy barns in New Jersey were reported by Hansens *et al.* (1955) and Hansens & Scott (1955). In the summer of 1955, these tests were continued in most of the same barns and with most of the same insecticides used in 1954. Residual sprays and wet and dry baits were compared under practical field conditions. Certain phases of the work were done in cooperation with the Geigy Agricultural Chemicals Company.

Two new phosphates, Dow ET-14 (0,0-dimethyl 0-2,4,5-trichlorophenyl phosphorothioate) and Dow ET-15 (0-methyl 0-2,4,5-trichlorophenyl phosphoramidothioate), as well as Diazinon, malathion, and Am. Cyanamid 4124 were tested as residuals and baits. Chlorthion was tested only as a residual, and Bayer L 13/59 only as a bait. Several commercial bait formulations with malathion as the toxicant were also tested.

Residual sprays were applied with power equipment, dry baits from shaker cans, and wet baits from sprinkling cans. All formulations were applied as necessary as determined by frequent counts. In the case of residuals, counts were made three times per week. With baits, counts were made five times per week. At each visit a fly index was determined, counts were made on six stanchions and on six scudder fly grids (18 inches square) and once a week sweeps were made with an insect net. These four counts were used in evaluating treatments. A total of 60 flies on six stanchions and six grids was regarded as failure to control flies. With baits effectiveness was evaluated by considering (1) the per cent of the counts where there were less than 20 flies on six grids and six stanchions, and (2) the average flies counted per day for the period of treatment. Details of methods used in treating barns and control evaluation are given by Hansens *et al.* (1955).

RESULTS WITH RESIDUAL SPRAYS.—Effectiveness of the various residual sprays is set forth in table 1, from which it is evident that in 1955 there was again considerable variation in effectiveness of any one insecticide, depending on breeding conditions and, in some cases, on materials used in barn construction. In the case of barn 16, particularly, which has a corrugated aluminum roof, all materials tested in the past 2 years have lasted a shorter time than in other barns.

Diazinon was by far the best material tested, giving 12 weeks or more of control when used at 1% concentration. At 0.5% emulsifiable solutions gave 7 to 12 weeks control. A mixture of Diazinon and butoxy polypropylene glycol (Crag fly repellent) was not as good as Diazinon alone in these tests. Diazinon was applied in four barns in early May and in these barns, since the early fly population was wiped out and the breeding potential reduced, particularly good results were obtained. In one barn sprayed May 29, 1954, control was obtained until August 1955, a period of more than 14 months. When laboratory-reared flies were exposed on the walls for 30 minutes in July 1955, kills of 27 per cent of the flies resulted.

Other materials which were used both years gave poorer results in 1955 than in 1954. Malathion with sugar generally gave 2 to 4 weeks control. Am. Cyanamid 4124 at 0.5 per cent and 1 per cent gave 3 to 6 weeks control and Chlorthion gave 4 weeks control at 0.5 per cent concentration and 7 weeks at 1%.

Dow ET-14 wettable powder, 0.5%, was superior to

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Table 1.—Fly control from residual insecticides in dairy barns.

Insecticide	Amount per 100 Gallons	Barn No.	Treated	Days Control
Diazinon, 25% W[a]	32 lbs.	1	May 7	133
		9	Aug. 24	23[b]
		11	July 26	51[b]
		22	May 5	90
		26	June 3	104[b]
Diazinon, 25% Em.	2 gals.	22	Aug. 24	23+
	2	25	June 3	84
	2	5	June 13	45
	4 gals.	2	May 7	110
		2	Aug. 25	22[b]
		5	July 29	49[b]
		9	May 7	108
		19	July 26	51[b]
Diazinon+Crag Fly Repellent,[c] 25% of ea. Em.	2 gals.	16	Aug. 10	28
		17	July 14	48
Malathion, 25% W.	40 lbs.+ 20 lbs. sugar	10	May 5	24
		10	June 20	28
		10	July 29	19
		10	Aug. 24	15
		15	June 20	42
		15	Aug. 10	35[b]
Malathion, 57% Em.	2 gals.+ 20 lbs. sugar	24	May 5	42
		24	July 29	17
		24	Aug. 24	22
Am. Cyanamid 4124, 50% W.	8 lbs.	14	June 20	37
		14	Aug. 10	36[b]
		17	June 16	21
	16 lbs.	44	Aug. 3	40
Am. Cyanamid 4124, 25% Em.	2 gals.	8	June 13	31
		44	June 20	25
		48	June 20	48
		48	Aug. 10	35[b]
	4 gals.	8	Aug. 3	37
Chlorthion, 25% W.	16 lbs.	12	Aug. 25	21[b]
	32	12	June 16	48
Dow ET-14, 25% W.	16 lbs.	6	June 13	65
		49	June 16	61
	32 lbs.	6	Aug. 24	23[b]
		49	Aug. 25	21[b]
Dow ET-14, 25% Em.	2 gals.	16	June 16	14
	4	16	July 14	18
Dow ET-15, 25% W.	16 lbs.	7	June 13	38
		50	June 16	32
		50	July 27	49
	32 lbs.	7	July 29	37
Dow ET-15, 25% Em.	2 gals.	3	June 13	35
	2	3	July 29	30
Dow ET-14 & Dow ET-15, 25% W.	8 & 8 lbs.	4	June 13	23
		18	June 20	21
	16 & 16	4	July 14	24
		4	Aug. 24	21[b]
		18	July 14	36
		18	Aug. 24	14

[a] W. refers to wettable powder, Em. to emulsion concentrate.
[b] Material still effective Sept. 15 when regular observations were stopped. A month later control was being obtained with Diazinon but not with the other materials.
[c] Butoxy polypropylene glycol.

0.5% emulsion. The wettable powder gave 8 weeks control. Dow ET-15 was not as effective. Four weeks of control resulted from using 0.5% emulsifiable solution and 4 to 6 weeks from wettable powder 0.5%.

Dow ET-14 and Dow ET-15 used in combination were less effective than when either was used separately. With 0.25% of each (wettable powders) about 3 weeks control was obtained. Using 0.5% concentrations, 2 to 5 weeks control resulted.

With both Dow ET-14 and Dow ET-15 used separately or together, few dead flies were found in the barns. Apparently kills were slow and flies moved from the barns before they died. At times flies were troublesome in barns at milking time but had disappeared 2 to 3 hours later.

Results with Baits.—Both wet and dry baits were usually applied in dairy barns five times per week to those areas of the barn floor where animals would not contact the baits. Results of bait treatments are summarized in table 2. Results are given in terms of (1) per cent of counts with less than 20 flies on six stanchions and six grids and (2) average flies on six stanchions and six grids per day during the period of treatment. Baits were considered unsatisfactory if this average was more than 60 flies. Variation in results was dependent on amount of bait used and local fly breeding conditions.

Dry baits of Diazinon and Bayer L 13/59 gave excellent results. Dow ET-14 and Dow ET-15 were not quite as good and there was little difference between these materials when formulated as dry sugar baits. Am. Cyanamid 4124 and Ortho dry bait were less effective than the Dow materials while malathion (1% on sugar) and Fasco fly flakes were unsatisfactory in our tests.

Among the wet baits, Diazinon was slightly superior to malathion and Bayer L 13/59. However, Diazinon was applied in two barns which had the highest fly breeding potential of any under test, and consequently the wet bait performed very well under bad breeding conditions. Especially in barn 24 the use of bait continued to reduce the fly population through the season even though the amount of bait used was reduced.

The wet baits gave results about equal to dry baits in 1955. Dry baits have the advantage of ease of application but the disadvantage that no residual is left when the barn is cleaned.

It was extremely apparent in 1955 that baits failed in control of *Stomoxys*. Especially from mid-July to September, a large portion of the flies counted in the barns treated with baits were *Stomoxys*. Where residuals were used, the number of stable flies was found to be much less.

Again in 1955 it was evident that residual treatments are superior to baits both in giving superior control of house flies and better control of stable flies. The better baits, however, gave satisfactory control where stable flies were not abundant and where the barn was reasonably clean.

Baits Compared by the "Plate" Method.—Several investigators have tested various fly baits by exposing the baits on plates or other containers in a barn and comparing effectiveness by counting flies visiting the baits or dead flies on the dishes. This method of comparing baits was tested on September 2 and 3 when 20 baits were compared in a heavily infested dairy barn. One ounce of bait was placed in an aluminum pie tin and spread evenly over the bottom. Pans were placed in a line about 2 feet apart and on the edge of the feed trough. Flies resting on the pans were counted 10 times each half hour for a 2-hour period and dead flies were counted at the end of the 2 hours. Thus 40 counts were made of flies attracted to baits during each of the 2 days. After each 10 counts, the pans were rearranged at random.

Baits included Diazinon, Bayer L 13/59, Am. Cyanamid 4124, Dow ET-14, Dow ET-15, and malathion, using 1% of the toxicants on sugar and 11 commercial formulations containing 1% or 2% malathion on a variety

Table 2.—Results from use of baits five times per week for fly control in New Jersey, 1955.

Treatment	Ounces of Bait Used per 1000 Sq. Ft.	Barn No.	Date Applied	Per Cent of Count Having Less Than 20 Flies	Grids and Stanchions Average Flies/Count
Dry Bait					
Diazinon, 1% on sugar	2	27	7/ 2 to 7/20	0	53
	4	37	7/21 to 9/ 1	57.1	21
	as needed	47	6/ 7 to 8/29	75.0[a]	15[a]
Malathion, 1% on sugar	2	51	6/15 to 6/27	0	98
	4	51	6/28 to 7/12	0	101
Malathion, 2% on hominy grits (Ortho Dry Bait)	4	34	6/22 to 7/ 6	55.5	44
	4	51	7/13 to 7/20	0	77
	6	51	7/21 to 9/ 2	10.7	61
	4	46	7/ 6 to 9/10	30.0[a]	50[a]
Malathion, 2% (Fasco Fly Flakes)	4	36	8/ 5 to 8/17	0	81
	8	39	7/18 to 8/ 3	0	143
Bayer L 13/59, 1% on sugar	3	43	6/15 to 9/ 2	20.0	41
Dow ET-14, 1% on sugar	3	35	8/ 4 to 8/17	0	61
	2	41	6/15 to 7/21	23.1	27
	3	41	7/22 to 8/ 3	88.9	14
Dow ET-15, 1% on sugar	2	35	6/16 to 6/28	0	81
	3	35	6/29 to 7/12	20.0	42
	2	35	7/13 to 7/20	16.7	53
	3	35	7/21 to 8/ 3	20.0	40
	3	41	8/ 4 to 8/17	75.0	12
Am. Cyanamid 4124, 1% on sugar	2.5	32	7/21 to 8/17	11.8	56
Wet Baits					
	Toxicant				
Diazinon, 25% emuls. sol.	1 oz.	39	8/ 4 to 8/18	77.8	26
	1 oz.[b]	39	8/22 to 9/ 2	0	37
	0.6 oz.	42	6/21 to 7/11	6.7	122
	0.3 oz.	42	7/12 to 7/18	0	62
	0.3 oz.[b]	42	7/21 to 8/26	13.0	49
Malathion, 57% emuls. sol.	0.3 oz.	39	6/22 to 7/12	6.7	68
	0.6 oz.	46	6/ 4 to 7/ 5	33.3[a]	49[a]
Bayer L/13/59 technical	2.5 gm.	34	7/7 to 7/26	0	92
	4.0 gm.	36	6/22 to 7/12	18.8	70
	2.0 gm.[b]	31	8/ 4 to 9/ 2	33.3	38

[a] Counts on stanchions only. Actual count on six stanchions doubled to equal stanchion plus grid counts.
[b] Three applications bait per week.

of base materials. These baits are labeled A to K in table 3. Sugar alone and the base formulation for bait G were also included in the tests and in addition in one test an empty dish was used. Totals for the two tests are given in table 3, which shows there was great variation between baits including the 11 commercial formulations.

It is noteworthy that only one bait attracted more flies than sugar and the best bait attracted only 42% as many flies as the base material for bait G. Apparently all toxicants except possibly Am. Cyanamid 4124 and Bayer L 13/59 were repellent to flies. Diazinon and Dow ET-15 on the other hand, attracted very few flies and killed even less. However, as stated above, in practical field tests Diazinon and Bayer L 13/59 were about equally good. On the other hand, Am. Cyanamid 4124, the best material in these tests, was one of the poorer formulations when used in treatment of dairy barns. Thus it seems that the "plate" method of testing baits does no give a true picture of the usefulness of baits, and, in ou experience, can lead to very misleading conclusions con cerning the value of a given bait formulation. In view o this, comparisons of commercial products by this method may also be misleading.

Tests were made in six barns with residual applications and baits together. The residuals were applied in June and baiting was begun when the residual treatment started to fail. In these tests, Diazinon, malathion, and Am. Cyanamid 4124 residuals and baits were used as well as Chlorthion residual with Bayer L 13/59 bait. In all cases the residuals and baits gave results comparable to those reported for these materials previously. Thus, Diazinon residual and bait gave excellent results. Chlorthion and Bayer L 13/59 were next best followed by Am. Cyanamid 4124 and malathion residuals and baits. In

Table 3.—Test of fly baits exposed in aluminum pie tins for 2 hours on each of 2 days.

	Total Flies Attracted to Bait[a]	Total Flies Dead in Pan
1% toxicant on sugar		
Diazinon	54	2
Bayer L/13/59	674	39
Malathion	174	7
Am. Cyanamid 4124	933	18
Dow ET-14	345	1
Dow ET-15	40	0
Commercial Formulations[b]		
A	249	37
B	435	87
C	100	12
D	359	32
E	227	24
F	377	86
G	295	12
H	152	5
I	441	20
J	206	25
K	134	2
Sugar	705	6
Base for Bait G	2226	6
Empty Dish	58[c]	1

[a] 80 counts, 40 each day.
[b] containing 1 or 2% malathion.
[c] 40 counts on 1 day.

general, two or more residual applications would give better fly control than the residual-bait combination. Cost of the latter would be less and might favor this method where the fly population was not large.

Studies of Milk from Diazinon-Treated Dairy Barns.—Before Diazinon can be used in dairy barns, it is essential to know whether the insecticide will be found in the milk or will alter flavor of the product after being used as a residual spray or bait. In cooperative tests with the Geigy Agricultural Chemicals Company, three barns were treated with Diazinon and milk samples were taken for chemical analysis and flavor tests. One barn was sprayed with 1% Diazinon applied as 32 pounds of wettable powder, 25%, per 100 gallons spray; the second was sprayed with 1% Diazinon applied as 4 gallons of an emulsifiable solution 25%, per 100 gallons spray; and the third was treated with 1% Diazinon on sugar applied 10 ounces per 1000 square feet daily during the period samples were taken. Treatments with residuals were made on July 26 and baiting was begun that day. Milk samples were taken before spraying and 1, 3, and 7 days after spraying. A composite milk sample was taken from the herd, bottled, cooled and then transported to the laboratory for analysis and taste tests.

The milk was analyzed by the sulfide procedure for the determination of Diazinon residues as modified for milk (Gigger 1955) and cholinesterase inhibition was determined by the method described by Cook (1954). These determinations were made by Richard P. Gigger of Geigy Agricultural Chemicals Company. Flavor studies were supervised by J. L. Horsfall.

These studies showed that there was no Diazinon in the milk in samples from any of the treated barns. The determinations of cholinesterase inhibition of milk from Diazinon-treated barns showed only the slight irregularities noted in day-to-day samples of milk and proved conclusively that there was no cholinesterase inhibitor in milk from barns treated with Diazinon. With respect to flavor, it was concluded that the sprays and bait did not affect the flavor of milk from cows brought into these barns for milking 24 hours after treatment.

Effect of Diazinon on Horn Flies.—On one farm in Salem County, in preparation for studies of sprays for control of biting flies, counts were made daily on the herd in the pasture. At the time these counts were being made, the barn was sprayed with 0.5% Diazinon as an emulsion. Daily counts of horn flies previous to the barn spraying averaged 98 per animal. The day after the spray was applied, the horn fly population dropped to four flies per animal. For 2 weeks thereafter the horn fly population remained at 18 per animal and no treatment of the herd for horn flies was necessary for nearly 2 months. Similar observations were made at other locations in both 1954 and 1955 but without "before and after" counts of flies.

These observations seemed to indicate that Diazinon was responsible for the reduction in horn flies even though the animals were in the barn for only 2 to 3 hours per day. A series of tests were then undertaken to elucidate this problem.

Special Studies of Diazinon.—When next a barn was sprayed, cages of house flies were exposed in the barn at intervals after treatment. On July 26, a barn was treated with 1% Diazinon (wettable powder) at 10:00 A.M. and flies were exposed in the barn at 3:00 P.M. in such a way that no flies could contact any sprayed surface. The cages consisted of 1 pint cylindrical paper cartons with the ends replaced by gauze. Flies were exposed for 30 minutes and additional cages were left in the milk house to serve as untreated checks. The milk house was connected directly to the barn but had not been sprayed. Further exposures of flies were made 6 and 21 days after spraying. Results of these tests, set forth in table 4, show that kills were large even though flies had no contact with treated surfaces. On the day of spraying, all the flies in the check cages kept in the adjacent, but unsprayed, milk room were killed. In all cages, most flies were down in 5 minutes.

On succeeding tests flies were knocked down more slowly and mortality also decreased. Check flies were placed in a nearby barn separated by about 50 feet from the sprayed barn on the 6- and 21-day tests.

Cattle were brought in for milking during the first test and at the end of an hour the fly population of 15 to 20

Table 4.—Exposure of flies in cages in a barn treated with 1 per cent Diazinon.

		30 Min. Exposure		Check	
Interval	Cage No.	Total Flies	Per Cent Mortality	Total Flies	Per Cent Mortality
Day of spraying	1	130	100	134[a]	100
	2	142	100	103[a]	100
After 6 days	1	91	95.6	68	0
	2	91	96.7	63	4.8
After 21 days	1	65	12.3	44	0
	2	74	6.8	59	0

[a] Exposed in milk house.

Table 5.—Flies exposed to panels placed in Diazinon-sprayed barn for 24 hours on days indicated.

Panel Exposed for 24 Hours	Laboratory Exposure of Flies	Mortality After 24 Hours: 6 Exposed Panels, Total Flies	6 Exposed Panels, Per Cent Mortality	1 Panel in Nearby Bldg., Total Flies	1 Panel in Nearby Bldg., Per Cent Mortality
Sept. 2, Side toward ceiling	15	188	34.6	31	3.2
	30	195	67.1	26	3.8
Side away from ceiling	15	164	15.2	36	5.5
	30	184	39.7	33	6.6
Check flies		29	3.4		
Sept. 5, Side toward ceiling	15	133	13.5	28	0
	30	177	40.7	26	0
Check flies		27	0		
Sept. 9, Side toward ceiling	15	—	—	—	—
	30	170	2.4	28	0
	60	180	11.7	31	0
Check flies		22	0		

biting flies (horn and stable) per animal had been reduced to 2 or 3 per animal.

These results seemed to indicate that Diazinon either killed by fumigation or deposited on the surface of the cages. It was then decided to expose unpainted plywood panels in a barn for 24 hours and then to test these panels in the laboratory to see if house flies could be killed. This was done when another barn was resprayed with 1% Diazinon on August 25 at 10:00 A.M. Panels were exposed beginning at 3:00 P.M. parallel to the treated surface and 3 inches from it for 24 hours and flies were then exposed on these panels in the laboratory for 15, 30, and 60 minutes. With 60 minutes of exposure, 35 per cent mortality was obtained on the side of the panel which faced the treated surface.

In a more extensive test a third barn was sprayed with 1% Diazinon wettable powder and panels were exposed for 24 hours. One set of six panels was exposed beginning 6 hours after the barn was sprayed. Other sets of panels were exposed for 24 hours each beginning 3 days and 7 days after spraying. All panels were exposed parallel to the ceiling and 3 inches from it. At the same time, cages of flies were exposed for 30 minutes by hanging them 8 inches from the ceiling on the day of spraying and again 7 days later. This exposure of flies resulted in 99% and 25% mortality, respectively.

Results of tests with panels, set forth in table 5, show that Diazinon transferred to panels exposed in the barn even when exposures were made a week after spraying the barn. With the passage of time, apparently the amount of Diazinon desposited on panels was less.

LABORATORY STUDIES OF DIAZINON.—Following the above-described tests a series of tests was conducted in the laboratory in which house flies were exposed to vapors from unpainted plywood panels treated with Diazinon wettable powders and emulsions. Panels were prepared with the aid of Geigy Agricultural Chemicals and by use of their automatic laboratory equipment whereby 150 mg. of actual Diazinon were applied to a square foot of plywood surface.

In these tests flies were exposed in embroidery hoop cages fitted with marquisette cloth. Exposures were made in two ways on panels treated with either wettable powders or emulsions. In one series, cages of flies were placed on a table and panels were placed with the treated side

Table 6.—Exposure of flies and cloth cages to Diazinon residues of 150 mg/sq. ft. on plywood panels.

Exposure[a]	Flies Exposed and Mortality After 24 Hours: Wettable Powder, *Total*	Wettable Powder, *Per Cent*	Emulsion, *Total*	Emulsion, *Per Cent*
Panel ½ in. above cages				
Flies & cloth	188	99.5	186	82.8
Flies alone	157	1.9	156	3.0
Cloth alone	86	98.9	87	95.4
Panel ½ in. below cages				
Flies & cloth	160	15.0	148	10.1
Flies alone	151	6.3	174	3.4
Cloth alone	91	41.8	81	18.5
Untreated	66	1.5		

[a] Exposed 60 minutes at 60° F.

down ½ inch above the cages. In the second series, the panel was placed on the table treated side up, and empty cages were placed on this treated panel. On these empty cages, a second group of cages was placed containing the flies to be exposed. Thus flies were ½ inch above the panel during exposure. Cages with flies were exposed for 60 minutes and then removed to clean cardboard.

After the exposure, a new cloth was placed in half of the cages and the cloths which were removed were prepared as new cages and previously unexposed flies were placed in the cages so prepared. Thus there were groups of flies exposed for 1 hour and kept in a clean cage, flies exposed 1 hour and kept on cloth exposed for 1 hour, and unexposed flies on cloths which had been exposed for 1 hour. All flies were fed and counts of mortality were made after 24 hours as shown in table 6.

The exposures were made at 60° F. and low kills were obtained on several treatments, probably because Diazinon was less volatile at low temperature but possibly also because flies were less active. In a preliminary test at 75° F. higher kills had been obtained. It was evident, too, that the majority of the kill in cages where flies were exposed above the panel was due to deposition of Diazinon on the cloth. This led to the conclusion that flies were not being killed when exposed above the panel because the cloth forming the floor of the cage was being removed when the flies were removed at the end of the hour exposure. This cloth was in the same position with regard to the treated panel as the one in the cage when flies were exposed below the panel. A further test was run and this time flies were also placed in this bottom cage after the exposure of 60 minutes on panels. In this series, flies and cloths were exposed to Diazinon for 15 and 60 minutes and the room temperature was 75° F. Results of these tests, reported in table 7, show that at 75° F. mortality was appreciably greater than at 60° F. Part of this higher mortality was apparently due to direct fumigation. Also, these data seem to show that much of the kill of flies is due to deposition of Diazinon on the cloth closest to the treated panel and not alone to fumigation. It made little difference whether this cloth was above or below a panel. It is obvious from these tests that flies need no direct contact with a surface treated with a

Table 7.—Exposure of flies to Diazinon residues of 150 mg/sq.ft. on plywood panels.[a]

	Flies Exposed and Mortality After 24 Hours			
	Wettable Powder		Emulsion	
	Total	*Per Cent*	*Total*	*Per Cent*
Exposure 15 minutes				
Panel ½ in. above cages				
Flies and cloth	134	100	156	89.1
Flies alone	155	45.2	142	30.3
Cloth alone	141	66.7	121	62.0
Panel ½ in. below cages				
Flies and top cloth	154	50.0	163	44.2
Flies alone	115	42.6	139	43.9
Top cloth alone	137	13.9	114	7.0
Bottom cloth alone	91	100	159	86.8
Untreated	65	7.7		
Exposure 60 minutes				
Panel ½ in. above cages				
Flies and cloth	96	100	101	100
Flies alone	79	97.5	47	78.7
Cloth alone	96	100	76	100
Panel ½ in. below cages				
Flies and cloth	86	97.7	90	84.4
Flies alone	72	91.7	67	82.1
Top cloth alone	36	75.0	81	76.5
Bottom cloth alone	67	100	53	100
Untreated	33	3.0		

[a] Tests at 75° F. in air conditioned room.

residual application of Diazinon but may contact it secondarily by its transfer to another surface or by contact with Diazinon vapors. In all of these tests wettable powder was superior to emulsifiable solutions.

Thus Diazinon apparently kills in barns by fumigation and by the insecticide transferring to other surfaces than those sprayed as well as by contact directly with sprayed surfaces. This may include the animals when they are in the barn and probably accounts for the observations that horn flies were controlled on dairy animals in the field even though the animals were in the barn only for about 2 hours a day for feeding and milking.

This seems to suggest, also, the possibility of spraying small areas in barns with this material at higher concentrations, possibly with fewer hazards to animals and certainly with lower labor costs for application. Likewise the effectiveness of Diazinon baits may thus be explained, particularly since flies, when they have a choice of materials in comparative tests, seem to avoid the bait.

Summary.—Tests of phosphate insecticides for house fly control were continued in New Jersey using both residual treatments and wet and dry baits. Diazinon was by far the best material tested. Other materials in order of effectiveness were: Dow ET-14 (0,0-dimethyl 0-2,4,5-trichlorophenyl phosphorothioate), Am. Cyanamid 4124, Chlorthion, Dow ET-15 (0-methyl 0-2,4,5-trichlorophenyl phosphoramidothioate) and malathion. Of the dry baits, Diazinon and Bayer L 13/59 were best. Dow ET-14, Dow ET-15, Am. Cyanamid 4124, and certain malathion formulations were also effective. Wet baits were about equal to dry baits but had the advantage of some residual action. The "plate" method of testing baits was found to be unsatisfactory for bait evaluation. Use of residual sprays supplemented by baits when the residual started to fail gave poorer fly control than additional residual treatments. Baits were not as effective as residual applications and failed to control biting flies.

Analysis of milk from herds housed in barns sprayed with Diazinon or treated with Diazinon baits showed that there was no Diazinon in the milk, no cholinesterase inhibitor, and no effect on flavor.

Horn flies were controlled for upwards of 2 months on herds housed in barns sprayed with Diazinon even though animals were only brought into barns for feeding and milking. Tests with house flies in sprayed barns and on unpainted plywood panels in the laboratory showed that flies are killed in part by fumigation and in part by direct contact with Diazinon. Also flies may be killed when they contact unsprayed surfaces on which Diazinon may deposit by transfer from sprayed surfaces.

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BULLETIN OF THE ENTOMOLOGICAL SOCIETY OF AMERICA

Members should read this publication carefully! It sometimes contains announcements, notices, etc., not sent to the membership in any other way.

Some Effects of Microwaves on Certain Insects which Infest Wheat and Flour

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This paper describes results of studies conducted by the Agricultural Engineering and Entomology Departments, Michigan State University, in cooperation with the Raytheon Manufacturing Company Research Laboratory, as part of an examination of the effects of radiant energy on biological tissues. The work reported was conducted on the effects of 12.25 cm microwaves on the granary weevil and the flour beetle.

It was brought out in another paper (Baker *et al.* 1955) that there is not enough energy in a quantum of a microwave frequency with wavelengths of about 0.25 cm. to 15 cm. to produce ionization in tissue. When microwave energy is absorbed in tissue or any dielectric loss material, the electromagnetic energy is changed into heat.

REVIEW OF LITERATURE.—It has been demonstrated by Clark (1950) and Herrick (1952) that the principal effect obtained by exposing various parts of animals, including eyes, internal body cavities, testicles, and muscular tissue to modest amounts of microwave energy, was a heating effect. Clark states that:

"I should like to emphasize again that this damage is entirely due to heat generated as a result of the absorption of microwave energy by the body tissue and is not due to any mysterious property of microwave radiation as such."

Most damage was to areas where proteins exist, such as in eye lens. Heat from microwaves seems to coagulate areas in the eye to form cataracts and the damage is not apparent immediately after exposure.

The work reported by Hines & Randall (1952), Richardson *et al.* (1950), and Salisbury *et al.*, (1949), point out some interesting effects of microwave energy on tissue. It was found that from studies made by Hines & Randall with 1600, 75, 12, 8 and 3-cm. radiations that the shorter electromagnetic waves were relatively more effective for increasing the temperature in superficial tissue than in deeper tissue and that longer wavelengths were more effective for heating of deep tissue than for superficial tissue. Tests were made to determine the effects of 12-cm. microwaves on the testes of adult rats. It was found that a single 10-minute exposure to microwaves caused testicular degeneration at a temperature of only 35° C. measured in the central area of the gland.

Salisbury *et al.* (1949) found that microwaves produce heat in the body where an abundant amount of blood is not supplied and also that blood is an effective coolant and acts to distribute heat developed evenly. It was pointed out that certain parts of the body are not effectively cooled by the blood. Examples include, lens in the eyes, gall bladder, parts of the intestines, and the testicles. When such organs are subjected to microwave irradiation, very high local temperatures may result. Richardson *et al.* (1950) found that when the flow of blood was sufficiently increased by continuous irradiation, the temperature ceased to rise and eventually was diminished when the rate of flow was great enough, so that the peak temperature often preceded the termination of irradiation.

Schwan (1953) proposed that the electromagnetic theory and information presented in the two papers by Schwan & Li (1953) and Schaefer & Schwan (1943) be combined into an equation which would give the field strength required to develop enough heat in insects in free space in order to have lethal effects. At the time of this writing a complete equation had not been fully developed using Schwan's suggestions; however, the equation presented in the next paragraph gives the relationship between the various factors involved.

The power necessary to raise the temperature of a given object in space, a distance r from an antenna in a given time, may be represented by the following formula:

$$WC(T_2-T_1)=4.2P(t)(e)(G)\frac{A}{r^2}$$

Solving for P:

$$P=\frac{WC(T_2-T_1)r^2}{4.2(t)(e)(G)A}$$

Where:

W = weight of object in grams
C = specific heat of object
T_2-T_1 = temperature rise in degrees Centigrade
4.2 calories = 1 Joule
P = power in watts
t = time in seconds
e = overall efficiency
G = gain of antenna = K/λ^2, where K is some constant depending on type of antenna
A = Cross sectional area of object being irradiated.
λ = wavelength
r = distance from object to antenna

It can be seen in the above equation that the power necessary to raise the temperature of an object in space with a given antenna is directly proportional to the square of the distance r and inversely proportional to the time t.

A specific problem is solved below using the following assumptions: an insect with a weight of 0.1 g, a specific heat of 1, an exposed cross sectional area of 0.1 sq. cm. is to be given a temperature rise of 55° C. in one second at a distance of 1 meter from the antenna. Assume also that the gain of the antenna is 10, and that the overall efficiency is 37 per cent. The overall efficiency of 37 per cent is obtained by multiplying $0.50\times0.75=0.37$. This assumes that the radio or microwave equipment is 50 per

PAID PAPER.

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Table 1.—Irradiation of *Tribolium confusum* Duv. adults and eggs with microwaves of a frequency of 2,450,000,000 cycles per second[a]

Sample No.	Exposure Time (Seconds)	Degrees F.	Dead 1 Day After Test	Additional Dead			Total Dead	Eggs Hatched
				2 Days After Test	3 Days After Test	After 1 Week		
1	3	90	2	0	0	0	2	121
2	3	90	0	0	1	0	1	115
3	3	90	1	1	0	2	4	143
4	6	108	17	0	2	0	19	139
5	6	108	20	0	1	2	23	149
6	6	108	29	2	0	2	33	119
7	9	129	50	—	—	—	50	98
8	9	129	48	1	0	0	49	83
9	9	129	50	—	—	—	50	75
10	12	154	48	0	0	0	48	60
11	12	154	50	—	—	—	50	72
12	12	154	50	—	—	—	50	42
13	15	165[b]	50	—	—	—	50	10
14	15	165	50	—	—	—	50	15
15	15	165	50	—	—	—	50	29
16	18	171	50	—	—	—	50	9
17	18	171	50	—	—	—	50	11
18	18	171	50	—	—	—	50	5
19	21	178	50	—	—	—	50	3
20	21	178	50	—	—	—	50	3
21	Check	—	1	0	0	0	1	142
22	Check	—	0	0	0	0	0	110
23	Check	—	1	0	0	0	1	137
24	Check	—	0	0	0	1	1	151

[a] Test conducted with a RADARANGE at the Raytheon Mfg. Co. Research Laboratory, Waltham, Mass. April 28–29, 1953. Fifty adult flour beetles were irradiated in one-half pint glass jars containing 75 grams of whole wheat flour.
[b] Estimated temperature.

cent efficient and that the efficiency of absorption is 75 per cent, i.e., 75 per cent of the incident energy is absorbed by the insect and 25 per cent is reflected.

The solution is:

$$P=\frac{.1(1)(55)(100)^2}{4.2(1)(.37)(10)(.1)}=35{,}000 \text{ watts}$$

If the distance (r) be increased to 5 meters, then the power required would be 880,000 watts.

If the antenna gain be unity, which may be the case for certain vertical antennas, the power required in the above two cases would be 350,000 watts and 8,800,000 watts, respectively.

Other values for t and G may be substituted in the equation and power requirements determined if desirable.

No experiments have been performed to evaluate the relative dangers or effects on insects or animal tissue of pulsed power as compared to continuous power. However, rough calculations by Salisbury *et al.* (1949) of the thermal time constants of typical physiological structures indicate that these are long as compared to the interval of a pulse of typical radar set. Accordingly, they point out that it would seem reasonable to evaluate the danger from apparatus of this type in terms of average power rather than peak power.

Salisbury *et al.* (1949) also reported that the field strength known to be dangerous to human beings is 3 watts per sq. cm., and is not likely to occur except in the immediate vicinity of a powerful transmitter. The area of a cross section of a typical 10-cm. wave guide is about 28 sq. cm. A power of about 90 watts is required to reach the danger level in this wave guide. In free space, the energy level is much less concentrated. Therefore, a much larger total power would be required to reach the danger point.

Apparently no information is available on the effect of microwave electromagnetic energy on insects. Although it has been fairly well predicted that any lethal effect of microwaves on insects would be due to pure heating effect, it was decided to conduct tests using microwave energy in order to determine possible lethal effects on insects.

EQUIPMENT AND PROCEDURE.—The data for the three tests conducted is recorded in tables 1, 2, and 3. Flour beetle adults, eggs and larvae were irradiated in the Radarange, Model 1132A, manufactured by the Raytheon Manufacturing Company, Waltham, Mass. A picture of a Radarange is shown in figure 1. This equipment operated on a frequency of 2,450,000,000 cycles per second employing the magnetron oscillator. The calibrated laboratory model of resonant cavity oven used for the tests was set at a power output (measured in a stand-

Table 2.—Irradiation of *Sitophilus granarius* (L.) adults and eggs with microwaves of a frequency of 2,450,000,000 cycles per second.[a]

Sample No.	Exposure Time (Seconds)	Degrees F.	Additional Dead				Total Dead After One Week	Number of Eggs Hatched
			1 Day After Test	2 Days After Test	3 Days After Test	1 Week After Test		
1	3	90	1	0	0	21	22	160
2	3	88	1	0	0	25	26	120
3	3	88	1	0	0	11	12	145
4	6	108	8	1	2	14	25	137
5	6	108	13	0	1	10	24	89
6	6	108	11	2	0	21	34	63
7	9	135	33	1	1	14	49	72
8	9	135	34	1	0	14	49	19
9	9	135	47	0	0	3	50	31
10	12	162	46	0	0	4	50	6
11	12	162	48	0	0	2	50	2
12	12	162	50	—	—	—	50	4
13	15	172	49	0	0	1	50	0
14	15	172	49	0	0	1	50	0
15	15	172	49	0	0	1	50	0
16	18	190	50	—	—	—	50	0
17	18	190	48	0	0	2	50	0
18	18	190	50	—	—	—	50	0
19	21	197	50	—	—	—	50	0
20	21	192	50	—	—	—	50	0
21	check	—	0	0	0	0	0	128
22	check	—	0	0	0	2	2	173
23	check	—	0	0	1	0	1	161
24	check	—	1	1	0	0	2	146

[a] Test conducted with a RADARANGE at the Raytheon Mfg. Co. Research Laboratory, Waltham, Mass., April 28–29, 1953. Fifty adult granary weevils were irradiated in one-half pint jars, containing 75 grams of Cornell Variety wheat.

ard 1-liter water load) of 940 watts. An absorption of the energy equivalent of this output would be considered maximum power transfer. Temperatures were taken on the samples, indicated in the tables, by a mercury thermometer, after exposure. Initial temperature of the samples before treatment was about 73° F.

Twenty-four samples were prepared for each of the three tests conducted. Fifty adult flour beetles were placed in each of 24 half-pint fruit jars (each containing 75 grams of whole wheat flour) about 4 days before the test date. During this 4-day period, the samples were kept in an incubator. This allowed sufficient time for the adults to oviposit before the test. This same procedure was used for the 24 granary weevil samples which were placed in 75 grams of Cornell 595 wheat in half-pint jars. The 24 samples of *Tribolium* larvae were prepared by placing a number of adults in a batch of whole wheat flour so that eggs would be deposited. After a period of incubation, the flour was mixed gently and divided into 24 samples of 75 grams each. To avoid injuring the larvae by handling them, no effort was made to place an equal number of larvae in each sample.

After the samples were prepared, they were shipped via air express to the Raytheon Research Laboratory, Waltham, Massachusetts. The air express company was requested to see that the temperature of the samples did not fall below 60° F. The samples were irradiated soon after they arrived at the Raytheon Laboratory. The samples arrived back in Lansing 4 days from the date of shipment from Lansing and were placed in an incubator. Then observations were made for lethal effects.

RESULTS AND DISCUSSION.—The data in tables 1, 2, and 3 indicate that a maximum temperature of 165° F. with an exposure time of 21 seconds in the Radarange was lethal to 100 per cent of adult flour bettles 1 week after treatment. Under these conditions 23 per cent of the eggs hatched. Two per cent of eggs hatched when exposed for 21 seconds at a temperature of 178° F. A temperature of 187° at an exposure time of 18 seconds was lethal to 100 per cent of the flour beetle larvae.

An exposure time of 15 seconds at a temperature of 172° F. was lethal to 100 per cent of the adult granary weevils 1 week after treatment. No flour beetle eggs hatched with this treatment. Only 2.5 per cent of the eggs hatched when exposed at a temperature of 162° F. for 12 seconds.

According to Dr. David Copson of the Raytheon Research Laboratory (in litt. June 3, 1953), the heat occurring in irradiated flour and grain is of high enough order to put these materials in the category of good absorbers of microwave energy. Since wheat and flour are good absorbers of microwave energy, the validity of a selective heating between the material and insects is questionable, because in each test conducted tempera-

Table 3.—Irradiation of *Tribolium confusum* larvae with microwaves of a frequency of 2,450,000,000 cycles per second.[a]

Sample Number	Exposure Time Seconds	Degrees F.	Total Dead Larvae 35 Days After Test	Living Adults Appeared 35 Days After Test
1	3	90	0	57
2	3	90	0	65
3	3	90	0	63
4	6	120	2	40
5	6	120	0	72
6	6	120	5	50
7	9	129	52	10
8	9	129	40	3
9	9	129	45	7
10	12	138	37	2
11	12	138	42	4
12	12	138	55	1
13	15	180	64	0
14	15	180	38	0
15	15	180	47	1
16	18	187	57	0
17	18	187	72	0
18	18	187	45	0
19	21	182	35	0
20	21	182	56	0
21	Check	—	0	60
22	Check	—	1	63
23	Check	—	0	75
24	Check	—	0	39

[a] Test conducted with a RADARANGE at the Raytheon Mfg. Co. Research Laboratory, Waltham, Mass., April 28–29, 1953. Flour beetle larvae were irradiated in one-half pint jars containing 75 grams of whole wheat flour.

tures approximating the lethal temperature found by other investigators was necessary before a 100 per cent kill was obtained.

Cost to Operate Magnetron.—Calculations in order to show the cost to raise the temperature of 1000 pounds of wheat per hour or flour from 70 to 140° F. are presented below (Schaefer & Schwan 1943):

70° (0.4 Sp. Heat) (0.00029 KWH/BTU) 1000 lbs/hr = 8.18 KW of radio frequency (RF) output required.

For an industrial application we may assume that magnetron oscillators for microwave generation would be available at a cost which would amount to $125 per 1000 KWH of RF output. Therefore the above flow rate for 1 hour would cost:

$$\frac{8.18\ (\$125)}{1000} = \$1.02 \text{ per hour for RF}$$

The electric power may be used for conversion to RF on a basis of about 60% efficiency. Thus,

$$\frac{8.18}{0.6} = 13.62 \text{ KW}$$

Fig. 1.—The Radarange showing control panel and oven—operating on an approved frequency of 2,450,000,000 cycles per sec., or a wavelength of 12.25 cm.

Estimating the cost per KWH at 2.0¢, this would mean 27.2¢ per hour and added to the cost for magnetron generators, it would be $1.29 per hour total. The further requirement would be for the machine or basic equipment and the cost of comparable output equipment from other suppliers to be a reasonable estimate. A rough estimate of $5000 for the equipment to do this calculated load may be made. The cost would then be on the order of 13¢ per 100 pounds of grain or flour irradiated.

Summary.—Tests were conducted using the Radarange with microwave wavelength output of 12.25 cm. When microwave energy reacts or is absorbed by matter most of this energy is changed into heat.

Wheat and flour are good absorbers of microwave energy. There does not appear to be any selective heating

action between the wheat or flour and the insects infesting these products because it was necessary to heat the total mass of wheat and flour to a lethal temperature before all insects in the test samples were killed.

A maximum temperature of 165° F. with an exposure time of 21 seconds in the Radarange was lethal to 100 per cent of adult flour beetles 1 week after treatment. Under these conditions 23 per cent of the eggs hatched. Two per cent of eggs hatched when exposed for 21 seconds at a temperature of 178° F. A temperature of 187° F. at an exposure time of 18 seconds was lethal to 100 per cent of the flour beetle larvae. An exposure time of 15 seconds at a temperature of 172° F. was lethal to 100 per cent of the adult granary weevils 1 week after treatment. No flour beetle eggs hatched with this treatment. Only 2.5 per cent of the eggs hatched when exposed at a temperature of 162° F. for 12 seconds.

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Laboratory Rearing of the Puss Caterpillar, with Notes on the Incidence of Parasitism[1]

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Since 1913, the larval stage of the puss caterpillar, *Megalopyge opercularis* (J. E. Smith), has been known for the severity of its sting. Bishopp (1923) reported that these caterpillars became so numerous around certain public schools in San Antonio and stings were so frequent that the schools were closed temporarily until the larvae could be brought under control. Both Foot (1922) and Bishopp (1923) have suggested that the principal effects of the sting are localized pain and dermatitis. More recently (Micks 1952) we have reported the occurrence of more severe reactions to the stings of puss caterpillars ranging from acute "shooting" pains to systemic reactions.

Little is known concerning the nature of the venom found in the poisonous spines of *M. opercularis* larvae. Foot (1922) noted that the local cellular reaction to the poison was chiefly lymphocytic. He found the toxic principle to be thermolabile and suggested that it may be combined with protein vehicles or may itself be a protein. Gilmer (1925), on the other hand, expressed the belief that the poison is probably not a protein but rather is adsorbed upon some protein molecule or group of molecules. In order to investigate the exact nature and mode of action of the toxic principle, it is necessary to have very large numbers of larvae. This presents a difficult problem not only from the standpoint of collecting sufficient numbers, but also because the majority of the larvae brought into the laboratory begin pupating before a sufficiently large number of them has been accumulated for venom collection. It is therefore the purpose of this report to present a simple method which was developed for rearing this species in the laboratory in large numbers and to report new information on the parasitism of this species.

MATERIALS AND METHODS.—Mature puss caterpillar larvae were collected in and around various kinds of trees and shrubs in the vicinity of Galveston, Texas. These were picked up gently with forceps and placed in temporary holding jars with covers until they reached the laboratory. They were then placed in battery jars (40 to 50 per jar) approximately 8½ inches high and 6½ inches in diameter (Figure 1). Each jar was covered with a piece of fine-mesh gauze held in place by a rubber band. Fresh yaupon leaves were added to the jars daily and the old twigs and dead leaves removed at least three times a week. Within approximately 2 weeks, all the larvae had pupated. About half of the cocoons were found attached to twigs with the remainder occurring around the sides of the jar, usually near the top.

Approximately 1 month later the first adult moths began to emerge. The adult females began to lay batches of eggs about 1 week after emergence, the number of eggs per batch varying from 25 to 120. These were laid in curved rows on the sides of the jar (Figure 2) as well as on the gauze cover. Covers containing eggs were removed and replaced by new ones to prevent crowding. Pieces of

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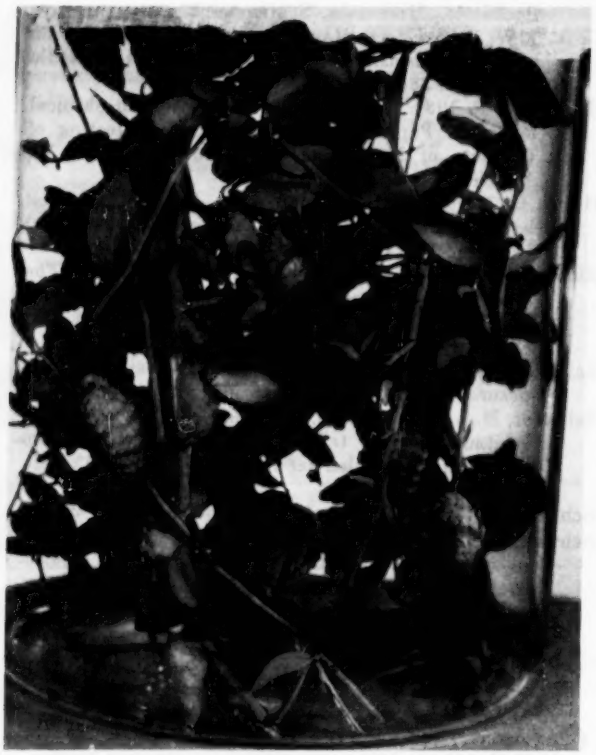

FIG. 1.—Rearing jar showing mature larvae and cocoons on yaupon leaves.

gauze from the original cover, containing separate batches of eggs were cut to facilitate placing them in clean jars (approximately 1000 eggs to the jar). As soon as the first larvae appeared, fresh yaupon leaves were placed in the jars daily. Old twigs and leaves were removed at least every other day, first examining them for the presence of the small larvae. These were disturbed as little as possible although it was frequently necessary to transfer some of them from dead to living vegetation with the aid of a camel's-hair brush.

FIG. 2.—Photograph through rearing jar showing characteristic clusters of eggs and newly hatched larvae.

At a room temperature of 25° C., the larvae reached maturity in about 6 weeks and began to pupate shortly thereafter. The entire life cycle, therefore, required approximately 3 months for completion. This makes it possible to rear through several generations per year. As with most other types of insects, the first larval stage is the most critical one. However, the mortality rate is rapidly reduced with each successive stage.

PARASITES OF *M. opercularis*.—The first five puss caterpillars collected by us several years ago were placed in a gauze-covered jar to which leaves were added until all specimens had pupated. Several weeks later, two flies were seen in the jar. One cocoon with an open operculum was closely examined and found to contain two empty fly puparia and a dried larval skin. The remaining four cocoons were opened and two of them contained four fly puparia each. One of these cocoons is shown in Figure 3. The adult flies were identified as *Carcelia lagoae* (Tns.), a species which has been previously known to be a parasite of *Megalopyge* larvae.

In order to obtain more information as to the natural incidence of parasitism of these caterpillars by tachinid flies, 624 larvae of *M. opercularis* were collected during November and December of 1954. These were supplied with fresh leaves daily until all had pupated. Six weeks after the first of the larvae had pupated, a live fly was seen in one of the jars. Within the next 2 weeks, all cocoons were opened and the flies which had emerged were collected and pinned for identification. A total of 123 of the cocoons, approximately 20 per cent, was parasitized by tachinid flies. *C. lagoae* was the predominant parasite although a number of flies belonged to a species of *Phorocera*, probably *claripennis* (Macq.). This species has been recorded as a parasite of several species of *Megalopyge*. The number of parasites per

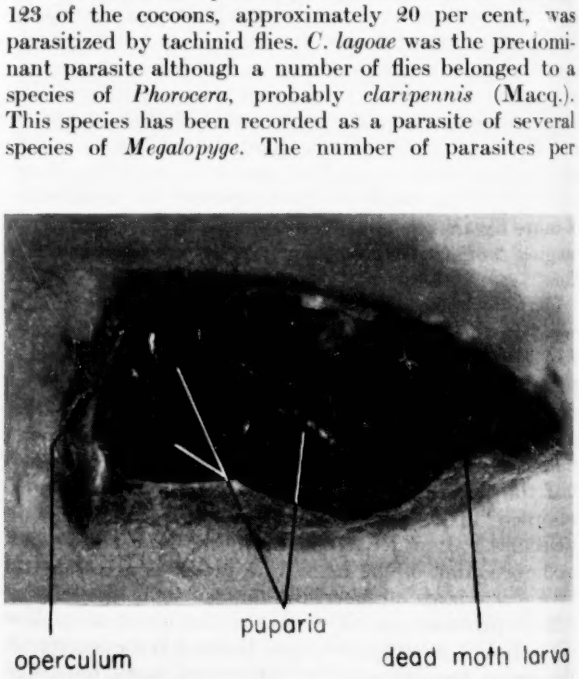

FIG. 3.—View of a cocoon with one side removed to show empty fly puparia and dead moth larva.

cocoon varied from one to five, with the majority containing three or four puparia. It was noted that when a cocoon contained four or more parasites, several of the adult flies which had emerged were usually malformed due to crowding. More rarely, the flies were unable to push open the door of the cocoon to escape. In general, the fewer the number of parasites occurring per cocoon the larger were the individual flies. Living fly puparia were found in a few cocoons as long as 2 months after pupation and one cocoon contained two living fourth-stage larvae 10 weeks later.

Aside from the species of Diptera already mentioned, the only other parasite found was an ichneumon wasp, *Compsocryptus retentor* (Brullé), which had emerged from one of the cocoons and was flying about in the jar.

SUMMARY AND CONCLUSIONS. A simple method was developed for the continuous laboratory rearing of the puss caterpillar, *Megalopyge opercularis*, through several generations per year. The entire life cycle was completed in approximately 3 months. A total of 624 larvae were collected to determine the natural incidence of parasitism. Approximately 20 per cent, 123, were parasitized by tachinid flies. *Carcelia lagoae* and a species of *Phorocera*, probably *claripennis*, were the predominant parasites. It would seem that these flies play a significant role in reducing the *M. opercularis* population in this area.

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Differences in Arsenic Tolerance Among the Sexes and Various Stages of the American Cockroach[1]

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The female of the species is generally the hardier sex and in this respect insects are no exception; for in addition to other points of superiority, the female is usually more tolerant than the male to a variety of insecticides. Heal (1942) found that arsenate, sodium fluoride, nicotine, pyrethrins, and rotenone were more toxic to the male American cockroach, *Periplaneta americana* (L.), than to the female irrespective of the method employed in the introduction of the poison (contact, ingestion, or injection techniques). Numerous other reports have been made concerning the superior tolerance of the females of various other species to one or more insecticides. It has also been found (Fink, 1926) that the young adults of *Leptinotarsa decemlineata* exhibit less tolerance towards arsenicals than do older individuals of the same species. In addition the tolerance of resistant-bred insect strains increases considerably more rapidly in the female than in the male. These facts suggest that, among other things, there is either a difference in the magnitude of detoxification mechanisms from sex to sex and from stage to stage, or a difference in the quality or quantity placed in the path of the toxicant so that in the former case less of the toxicant reaches the site of action and in the latter instance the rate of travel in the body is sufficiently retarded to reduce the concentration of the active material at the locality or localities of toxic action at any given instant. However, regardless of the mechanisms involved, the fact remains that differences in tolerance to insecticides exist within a species, and it is supposed that these differences are the reflections of certain morphological, biochemical, or physiological variations among the stages and sexes. There can be little doubt that the detection of such variations will contribute in some part to our understanding of the mechanisms of action of insecticides. The American cockroach was selected as suitable media for this investigation because of its large size and voluminous literature references.

METHODS.—*Injection of Arsenic Trioxide.*—Solutions of arsenic trioxide were injected into the thoracic cavity of CO_2-immobilized cockroaches by means of a micro-injection apparatus. All insects were deprived of food and water during the 24-hour period prior to treatment.

Reduced Glutathione (GSH) Determinations.—GSH was measured manometrically according to the procedure of Woodward (1935) and Behrens (1941) with several modifications (Forgash 1952). However, since the last communication additional modifications have been made. It is extremely important to obtain glyoxalase preparations which are low in GSH and high in enzyme activity. The following procedure proved most satisfactory since preparations were obtained which possessed high activity, prolonged stability, and relatively little GSH. One pound of fresh baker's yeast was divided into approximately equal portions, and each portion was mixed for 30 seconds with 400 ml. of c.p. acetone in a Waring blendor. The entire mixture was then placed in a large beaker, stirred for 5 minutes, and suction-filtered. The residue was blended with 500 ml. of acetone for 30 seconds and suction-filtered. To remove GSH from the yeast the residue from the last acetone wash was suspended in 600 ml. of glass-redistilled water and centrifuged in 200 ml. vessels. The washing process was repeated four times. With the equipment available this part of the procedure did not lend itself too well to blending; for a foamy suspension was obtained which greatly increased the

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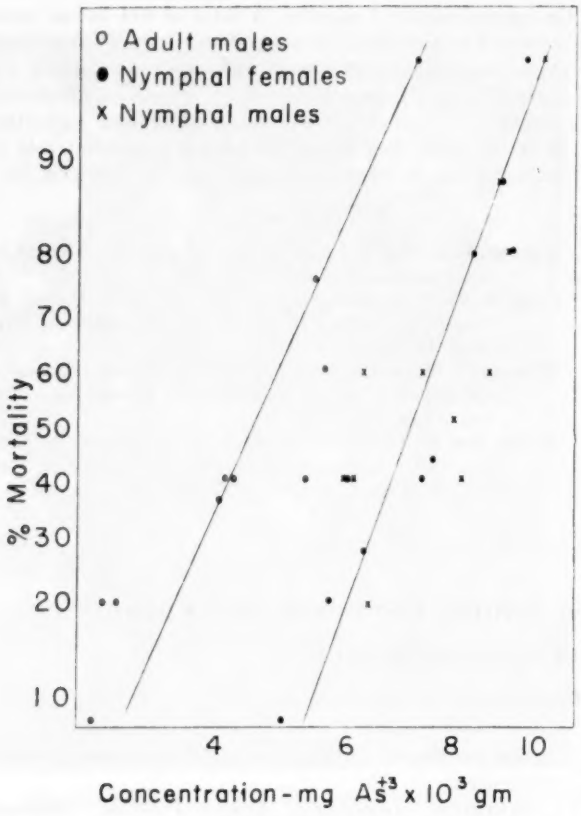

FIG. 1.—Comparison of the toxicity of arsenic trioxide to adult male and nymphal American cockroaches.

volume of the product, and thus lengthened the washing period. Whenever larger centrifuge vessels are available, use of a blendor will increase the efficiency of this step. After the yeast was washed free of GSH, the residue was blended three times with 500 ml. portions of c.p. acetone for 30 seconds and suction-filtered each time. The residue from the final acetone wash was blended with 300 ml. of c.p. ether for 30 seconds, suction-filtered, and spread in a thin layer on absorbent paper to dry for a period of 15 to 20 hours.

GSH was extracted from cockroach tissues according to a procedure previously described (Forgash 1952), with one exception. Previously a 2 to 4 hour extraction period had been employed with no significant differences in GSH values. However, it has since been found that higher values are obtained when the mixture of cockroach tissue and 2% sulfosalicylic acid is stored in the refrigerator overnight (16 to 18 hours) before filtration.

Respiration Measurements.—Oxygen consumption was measured in a Warburg respirometer, using vessels with an approximate capacity of 120 cc. The insects were anesthetized with CO_2 and weighed. After weighing, the insects were placed one to a flask and were permitted to recover from the effects of the gas before performing the measurements. On recovery, the flasks were placed in a 25° C. bath and shaken for a period of 15 to 20 minutes (100 oscillations per minute) to obtain temperature equilibrium and permit the insects to adjust to the unusual environment. The first reading, taken 5 to 15 minutes after closing the system, was always discarded since it often differed considerably from subsequent readings. During prolonged tests the air supply in the flasks was renewed at intervals by opening the system for 15 minutes. All measurements were made at 25° C. in a gas phase of air. Oxygen consumption was expressed in cubic millimeters of oxygen per gram of dry tissue per hour.

Table 1.—Comparison of the toxicity of arsenic trioxide to adult male and nymphal American cockroaches.

Adult Males		Nymphal Females		Nymphal Males	
Dosage	Mortality 96 Hrs.	Dosage	Mortality 96 Hrs.	Dosage	Mortality 96 Hrs.
mg. As $\times 10^3$/gm.	Per Cent	mg. As $\times 10^3$/gm.	Per Cent	mg. As $\times 10^3$/gm.	Per Cent
2.98	8	5.00	8	6.00	40
3.01	20	5.77	20	6.12	40
3.16	20	6.00	40	6.25	60
4.23	36	6.31	28	6.51	20
4.28	40	7.36	40	7.35	60
4.38	40	7.57	44	8.10	52
5.34	40	8.56	80	8.18	40
5.56	76	9.43	88	8.92	60
5.70	60	9.60	80	9.40	88
7.20	96	9.77	96	9.42	80
				10.43	96

EXPERIMENTAL.—*Comparison of the Tolerance of Nymphal and Adult Cockroaches to Arsenic Trioxide.*—Nymphs were selected from several cages which contained some mature individuals in order to obtain relatively mature nymphs. The nymphs were divided according to sex and the smaller individuals were discarded. Injection of arsenic trioxide was accomplished as previously described using 10 to 25 individuals for each dosage level of toxicant. Immediately following injection each group was

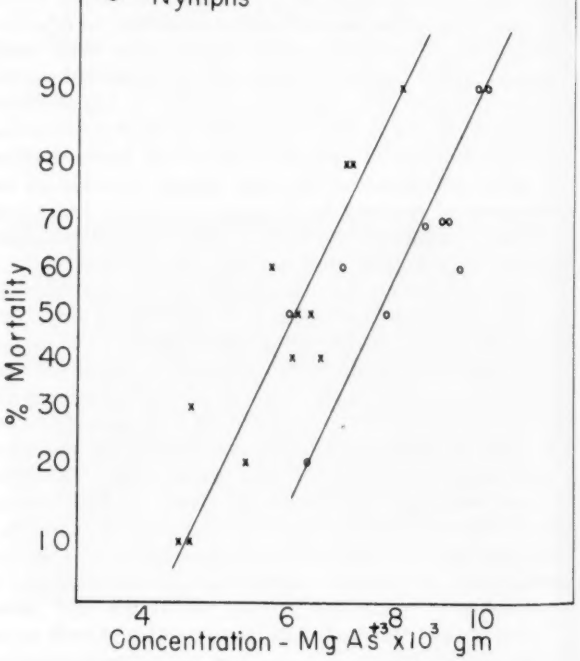

FIG. 2.—Comparison of the toxicity of arsenic trioxide to adult female and nymphal American cockroaches.

weighed and placed in battery jars for a period of 4 days, during which time mortality counts were taken at 24-hour intervals. The average weight per insect for each group was used to calculate the amount of arsenic per unit weight delivered to each insect in their respective groups. Comparable numbers of adult males were treated in an identical manner, and the tolerances of the two stages were compared. The data presented in table 1 are the collective results of tests conducted on four separate occasions.

The toxicity of arsenic trioxide to nymphs and female adult cockroaches was determined in the manner previously described. However, since there appears to be no difference in tolerance between the nymphal sexes (Figure 1), the nymphs were not treated separately according to sex, but rather five individuals of each sex were included in each treatment. The results of three separate tests are shown in table 2 and figure 2.

The order of increasing tolerance to arsenic trioxide of different stages and sexes of the American cockroach is: male adult, female adult, nymphs (both male and female).

GSH of Adults and Nymphs.—The forces of detoxification apparently differ in either magnitude, or kind, or both in the various stages and sexes of the American cockroach, since the arsenic tolerance varies from group to group. It has been reported (Forgash 1952) that arsenic attacks the GSH of the cockroach and that GSH injections will reduce the toxicity of arsenic. Measurements of the total GSH of male and female adults showed no apparent differences between the adult sexes (unpublished data), and therefore the superior tolerance of the female can not be attributed to a greater supply of GSH. Since it does not necessarily follow that such is the case with immature individuals, analyses were made of the GSH content of nymphs. The results of these tests are shown in table 3.

The variation in arsenic tolerance between the adult and immature sexes cannot be accounted for by differences in GSH concentration; for the nymphs, which are more tolerant, contain a slightly lower concentration than the adults (Table 3).

GSH of Nymphs at Different Periods within an Instar.—

Table 2.—Comparison of toxicity of arsenic trioxide to adult female and nymphal American cockroaches.

Adult Females		Nymphs (Mixed Sexes)	
Dosage	Mortality 96 Hrs.	Dosage	Mortality 96 Hrs.
mg. As $\times 10^3$/gm.	Per Cent	mg.As $\times 10^3$/gm.	Per Cent
4.54	10	6.06	50
4.69	30	6.36	20
4.69	10	6.96	60
5.40	20	7.87	50
5.83	60	8.71	70
6.13	40	9.08	70
6.21	50	9.08	70
6.43	50	9.69	60
6.59	40	9.92	90
7.04	80	10.30	90
7.12	80		
8.18	90		

Table 3.—The GSH content of last-instar nymphal American cockroaches.

GSH Content		
Male Adult	Female Nymph	Male Nymph
Mg. GSH per gram insect (dry weight)		
1.27	1.14	1.10
1.30	1.29	1.19
1.32	1.34	1.04
1.30 (average)	1.26 (average)	1.11 (average)

It has been reported (Ministerio De Agricultura 1936) that the application of sodium arsenite to various parts of the body of nymphal locusts, *Schistoserca paranensis*, produced lower mortalities if made just prior to moulting. Explanation of this phenomenon may lie in the fact that the cuticle thickness is greatest at this time, or possibly there are changes in the physical or chemical properties of the cuticle, such as an increase in waxy or oily secretions, which inadvertently protects against the action of water-soluble toxicants. However, there is also the possibility that just prior to the moult there is an elaboration of substances, such as GSH, or other sulfhydryl-containing substances, for the purpose of effecting moulting, and these substances react with the arsenic thus reducing the effective dose. Nymphs which were completing their final ecdysis were collected from rearing cages. So that sufficient material was available for the tests it was necessary to group both males and females in a single sample. The GSH of these newly-moulted adults was measured as previously described. A sample of older male adults was used in each test for comparative purposes. The results of these tests are shown in table 4.

Since no increase in GSH concentration was found in cockroaches which had just completed a moult, it was decided to investigate the content of nymphs which were approaching ecdysis. A difficulty arose in distinguishing individuals which were relatively advanced in their present instar. In view of the fact that the cuticle of the cockroach darkens slowly after ecdysis, the nymphs were segregated according to the degree of darkness of the cuticle. Those lightest in color were placed in one group and those darkest in color in another. To obtain as sharp a differentiation as possible those nymphs were discarded which appeared intermediate to the two extremes. The GSH concentrations of the two groups were determined and compared (Table 5).

Although there appears to be little difference in GSH concentration of nymphs in different stages of an instar (Table 5), the possibility remains that the GSH level does

Table 4.—The GSH concentration of newly-moulted adult American cockroaches.

No. and Sex of Cockroaches			GSH Concentration Relative to Older Adult Males
Male	Female	Total	
			Per Cent
6	6	12	63
9	3	12	68
			66 (Average)

Table 5.—The GSH concentration of nymphal American cockroaches which appear to be in different stages of an instar.

Test Group	Relative Difference in GSH Concentration
	Per Cent
Light-colored nymphs	+ 6
Dark-colored nymphs	− 6
Light-clored nymphs	−13
Dark-colored nymphs	+15

not increase gradually or uniformly over an instar, but increases rapidly over a short period immediately prior to the moult.

Rate of Metabolism of Adult and Nymphal cockroaches.—Fink (1926) has found that young insects of the species *Leptinotarsa decemlineata* are more susceptible to arsenical poisoning than are older individuals. Since it was found that the respiration rate of the former is greater than that of the latter, Fink suggested that the degree of susceptibility may depend on the metabolic rate. With the American cockroach it has been found that a given dose of arsenic is equally toxic to either sex of the last-instar nymphs, whereas a considerably lower amount is required to kill the male adult, and a slightly smaller quantity proves lethal to the adult female. If, as Fink suggested, the rate of respiration and the degree of susceptibility to arsenical poisoning are related, we might expect that the oxygen consumption rates of the nymphal sexes are equal or nearly so, and that the rates of the adult sexes are correspondingly higher than that of the nymphs. To test this premise the respiration rates of 15 to 21 individuals of each group were measured as previously described. The results of these tests are shown in table 6.

Discussion.—There is a difference in the tolerance to arsenic trioxide of different developmental stages and sexes of the American cockroach. Whereas the female adult is able to tolerate larger doses of arsenic than is the male, no such difference exists between the sexes of last-instar nymphs. In addition the last-instar nymph is more tolerant than either adult sex. The reasons for these differences are not understood, but there can be little doubt that they have some physiological basis. It has been demonstrated (Fink 1926, Forgash 1952) that arsenicals will react with the GSH of insects. If we assume that GSH functions as a protector for vital sulfhydryl enzymes (as has been repeatedly suggested) we may wonder concerning the existence of differences in GSH concentrations among the various stages and sexes. However, when this possibility was given experimental consideration it was found that the variations in tolerance are not accountable to differences in GSH concentrations, for the total body concentration is similar in the adult sexes and slightly lower in the nymphs.

Table 6.—The oxygen consumption of different stages and sexes of the American cockroach at 25° C.

Stage	Sex	No. of Insects	Oxygen Consumption		
			Range	Average	Standard Error
			cmm. O_2/gm. dry wght./hr.		
Adult	Male	21	1000–4680	2124	±936
Adult	Female	15	800–2420	1407	±380
Last-instar nymph	Male	16	775–2170	1277	±363
Last-instar nymph	Female	15	790–2105	1210	±373

Although arsenic trioxide does not have the immediate or complete effect on respiration of the cockroach as do cyanide and other respiratory poisons, it is of interest to note that the differences in tolerance closely parallel differences in respiratory rate as might be expected. There is considerable variation in respiration rate even among individuals of a given sex and developmental stage, just as there is a difference in individual susceptibility to a given toxicant. However, taking the average rates, it is found that male and female nymphs, which respond similarly to a given dose of arsenic, respire at equal rates; whereas the adult male, which is considerably less tolerant to arsenic, exhibits a higher degree of respiratory activity. The female adult respires at a slightly higher rate than the nymphs and at a considerably lower rate than the male adult, and is less tolerant to arsenic than the nymphs and more tolerant than the male adult. To suggest that the degree of susceptibility within a species varies directly with the rate of metabolism would be tantamount to saying that in the more rapidly metabolizing individuals the poison is transported more quickly to the site of toxic action with no compensative increase in the amount detoxified over a given period of time. This may or may not be the case. Presumably the detoxification mechanisms are in balance with the rest of metabolism and at a level corresponding to the overall metabolic rate of the individual. However, this has not been proven, and at best it may be true only within narrow limits. Be that as it may, the relationship between respiratory rate and degree of tolerance is a very interesting one and appears highly worthy of further investigation.

It is of interest to note that the difference in GSH content between newly-moulted adults and older adults is greater than that between last-instar nymphs and older adults. Possibly the GSH supply suffers depletion in the process of detoxification of waste products which accumulate during the change from nymph to adult, or, if GSH is an intermediate in protein metabolism, as Waelsch & Rittenberg (1941) suggest, the GSH reserves may be lowered as a result of the accelerated synthesis of tissue just prior to ecdysis.

Summary and Conclusions.—Male adult American cockroaches are much more susceptible to poisoning from arsenic trioxide than are female adults. Last-instar nymphs of both sexes exhibit similar degrees of tolerance to this arsenical and are less susceptible than the adult sexes.

Glutathione (GSH) is present in approximately equal concentrations in both male and female adults, and in slightly lower concentrations in last-instar nymphs. Thus the differences in arsenic tolerance cannot be explained on the basis of gross differences in GSH. However, it remains to be shown whether the distribution of this sulfhydryl in various tissues is constant from sex to sex and from stage to stage.

Last-instar nymphs were separated into two age groups for the purpose of determining the relative levels of GSH

in each group. Although only slight differences were observed, the possibility remains that differences exist within the groups themselves.

Cockroaches which have just completed their final moult contain approximately 30% less GSH than do older adults. This deficit may be the result of detoxification of toxic waste products or acceleration of protein synthesis in which abnormal amounts of GSH are utilized.

The respiratory rate of the male adult is considerably higher than that of the female adult, whereas both sexes of last-instar nymphs respire at the same rate which is less than either adult sex.

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The Distribution and Metabolism of DDT Injected into the American Cockroach[1,2]

D. G. COCHRAN[3]

The problem of insect resistance to insecticides has been attacked from a number of standpoints and, as evidenced by the amount of information which is currently available, this is highly advantageous. Among the insecticides which have been studied in this connection, DDT has probably received the most attention. In spite of this, much still remains to be learned about this insecticide. For example, little information is available on the disposition of DDT that is injected into the blood stream of an insect. Likewise, the existing knowledge on where DDT is metabolized within the insect body is very limited. These are but a few of the problems which still remain unsolved. It is the purpose of the present paper to report on an investigation dealing with several of these problems. Specifically, the following points have been studied using the American cockroach *Periplaneta americana* (L.), as the test species: (1) the distribution of DDT when injected into the haemolymph; (2) the metabolism of injected DDT in the various tissues, and (3) differences in the distribution and metabolism of DDT which might be related to the sex or to the developmental stage of the individual.

REVIEW OF LITERATURE.—The distribution of insecticides within the bodies of insects depends to some extent upon the method by which they are applied. When DDT was applied topically Sternburg & Kearns (1950) found that it occurred inside the body of house flies only in the cuticle-hypoderm. Similarly, when they fed DDT to the flies none was found anywhere except in the alimentary canal. Tahori & Hoskins (1953) have disputed these findings and have reported that they found DDT in all tissues tested after flies were treated topically with this insecticide. They do agree, however, that most of the DDT is in the cuticle-hypoderm. This position is supported by the findings of Lindquist *et al.* (1951) who mentioned specifically that they found DDT in the body fluids, gut, ganglia, reproductive system, and muscles as well as in the cuticle-hypoderm. In addition, Sternburg & Kearns (1952a) reported that the insecticide rhothane can be recovered from the tissues of topically treated red-banded leaf roller larvae.

Zeid *et al.* (1953) have presented data on the distribution of radioactive pyrethrins in the American cockroach. The method of application of these materials was primarily by interspiracular perfusions. From these studies it was found that radioactivity occurred in the fore-, mid-, and hind-intestine, brain, thoracic ganglia, thoracic muscle, and abdominal fat. Of these tissues the fore-gut contained the greatest amount of radioactivity. However, from $\frac{2}{3}$ (male) to $\frac{3}{4}$ (female) of the dosage of radioactivity applied was retained in the carcass of the insect.

In another group of experiments Morrison & Oliver (1949) injected radioactive arsenic trioxide into the haemolymph of last-instar *Tenebrio* larvae. Within 1 hour they found radioactivity throughout the haemolymph, in the body wall, fore-, mid-, and hind-gut, and Malpighian tubules. This radioactivity showed little change in distribution over a period of 20 hours although most of the larvae were still alive.

From this information it appears that at least the four insecticides mentioned are distributed quite generally in the insect body with some possible restrictions due to mode of application. This is true even though three of

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[2] This work was carried out while the author was a T. J. Headlee Research Fellow at Rutgers University, and is a part of the doctorate thesis submitted to the graduate faculty in partial fulfillment of the degree requirements.

[3] The author, who is now in military service, wishes to express his appreciation to Doctors B. B. Pepper and A. J. Forgash of the New Jersey Agricultural Experiment Station for their aid and suggestions throughout the course of this work, and to Dr. J. M. Grayson of the Virginia Agricultural Experiment Station for his assistance in reviewing and criticizing this manuscript.

the four are only slightly soluble in water and presumably also in haemolymph.

With regard to the sites of metabolism of insecticides in insects, it appears that for DDT the cuticle-hypoderm and the alimentary canal are the most important at least in house flies. It has been shown that in these tissues DDT is metabolized to DDE and possibly other products (Tahori & Hoskins 1953, Sternburg & Kearns 1950, 1952a). No information was found on this subject when DDT was injected into the haemolymph of house flies. This is also true for DDT injected into roaches although toxines (not DDT) were found in their blood (Sternburg & Kearns 1952b, Butts *et al.* 1953). One fragment of information is available on a site of detoxification in the American cockroach. Forgash (1952), using injected doses of arsenic trioxide, reported that the fat body reacted with a considerable amount of arsenic and he suggested that because the female has more fat body than the male this might be one reason for females surviving larger doses than males.

It is evident from the literature that sex and developmental stage play a significant role in determining the susceptibility of the individual insect. This information is derived from several insect species. For example, it is known that female house flies are less susceptible to DDT than are males in both resistant and susceptible strains (Barber & Schmitt 1948, Bruce & Decker 1950, Harrison 1952, Busvine 1951). Likewise, Grayson (1951) found the same situation in resistant and susceptible strains of the Germain cockroach, *Blattella germanica* (L.). In addition, Pielou & Glasser (1952) have shown that in the parasitic wasp, *Macrocentrus ancylivorus* (Hymen), females became resistant faster than males.

In an investigation dealing with some of the older insecticides, Heal (1942) found that with the American cockroach the adult female is less susceptible than the male. Munson & Gottlief (1953) obtained similar results with DDT.

When the role of both sex and stage of development was investigated, Ricci (1948) showed that female adults and male and female nymphs of the oriental cockroach, *Blatta orientalis* (L.) are all less susceptible to DDT than adult males. Similarly, Cochran (1955) found that the adult female and the last-instar male and female nymphs of the American cockroach exhibited approximately the same degree of susceptibility to DDT and were all less susceptible than the adult male.

EXPERIMENTAL PROCEDURE.[4]—The toxicant was applied to the cockroaches in the form of measured doses injected into the blood stream of the thoracic cavity. These injections were accomplished by means of a micro-injection machine equipped with a 27-gauge hypodermic needle. In each case adult males were injected with a dose of 0.002 cc. of solution, adult females with 0.004, and male and female last-instar nymphs with 0.003 cc. The amount of toxicant applied was varied by employing solutions of different concentrations. The solvent used to carry the toxicant was a mixture of 10 parts of acetone, 10 parts of xylene, 75 parts of deobase and 5 parts of absolute ethyl alcohol. DDT is completely soluble in this mixture at all of the concentrations employed in these experiments. Insects injected with only the solvent mixture were used as controls. The treated roaches were kept in battery jars in a constant temperature room (22° C.), and drinking water was provided in these jars in small containers. In these experiments only those insects which survived the tests were used.

The preliminary preparations for the dissection of tissues were begun by removing the roaches from the post-treatment containers and anesthetizing them with CO_2 gas. Without allowing them to recover from the effects of this gas the cockroaches were placed in a refrigerator and cold immbolized. In this condition they were stored until needed for dissection (usually less than 1 hour). It was found that the dissection was facilitated if the head and legs were removed. After this a mid-ventral incision was made down the entire length of the body. By pinning the insect to a paraffin-bottomed dissecting dish and spreading the cut ventral body wall it was possible to remove the desired tissues quite easily, with the exception of the brain and nerve cord, which were obtained from a dorsal dissection. The dissected tissues were immediately frozen and were stored in that condition. For purposes of this work the tissues were classified as follows: fat body, gonadial tissue (including gonads and accessary glands), Malpighian tubules, alimentary canal, thoracic muscles, brain and nerve cord, and remaining tissues. This last designation included all portions of the cockroach body not examined separately. As the tissues were removed from the insect they were rinsed in acetone to remove any DDT which may have been present in the adhering haemolymph. Analyses for DDT in the acetone rinse were negative.

For the extraction of insecticide the frozen tissues were thawed and placed in an ordinary glass test tube. Using a snugly fitting, ground-glass pestle, the tissues were thoroughly macerated in 10 ml. of benzene and the resulting extract was poured off into a 50 ml. Erlenmeyer flask. This procedure was repeated twice and all of the extracts were combined. Experience proved that further extraction was unnecessary. Likewise, filtering the benzene extract was found to be of no value. This method of extraction was used on all of the tissues except those designated as remaining tissues, which were placed in a Waring Blender with 100 ml. of benzene and ground for 1 minute. The ground material was then poured through a filter consisting of four layers of surgical gauze and a #1 Whatman filter on a Buchner funnel. The residue was rinsed several times and the various extracts combined.

All of the extracts obtained by the techniques mentioned above were evaporated to dryness and analyzed by the Schechter-Haller colorimetric procedure (Schechter *et al.* 1945, 1947). A Beckman Model DU quartz spectrophotometer was used for the measurement of the characteristic colors. These colors were developed in 3 ml. of benzene and 6 ml. of sodium methylate. No attempt was made to separate the alkali-insoluble metabolites from DDT and they were determined in mixture by the method of Knudson *et al.* (1940), using wavelengths of 596 mμ and 530 mμ. The identification of alkali-insoluble metabolites was not attempted as that was not of major importance to this problem. It was assumed that DDE

[4] DDT was supplied by Geigy Company, Inc.; DDE through the courtesy of C. W. Kearns, Department of Entomology, University of Illinois, Urbana.

Table 1.—Mortality produced by various concentrations of DDT 4 days after it was injected into adult male and female American cockroaches.

Dosage—μg. DDT	Per Cent Mortality	
	Males	Females
2	17.3	—
4	46.6	—
6	53.3	—
8	76.0	10.8
10	86.1	—
12	—	21.1
20	—	56.5
32	—	80.1
40	—	78.3

Table 2.—Mortality produced by various concentrations of DDT 4 days after it was injected into adult females and last-instar male and female nymphs of the American cockroach.

Dosage—μg. DDT	Per Cent Mortality		
	Adult Female	Female Nymph	Male Nymph
10	19.4	20.2	19.0
20	33.3	—	—
30	50.0	33.3	46.2
50	—	62.0	50.0
70	68.0	68.0	70.0
90	88.8	90.4	87.5

constitutes the major portion of these metabolites and hereafter they will be referred to as DDE. No alkali-soluble metabolites were found in any of the tissues tested. Interference from the color endogenous in the tissue extracts was overcome by making appropriate subtractions from the final readings.

RESULTS.—The 4-day, LD_{50} values for injected doses of DDT in the American cockroach are indicated by the data in tables 1 and 2. Each dosage was replicated 3 to 5 times with each replicate consisting of 20 to 25 insects. It can be seen that these values are approximately as follows: 5μg for adult males, 20 μg for adult females, and 35 μg for adult females and last-instar male and female nymphs. The two values given for adult females were obtained at different times and serve to illustrate the variation of the means of test groups that normally occurs. When the LD_{50} values were calculated in terms of μg of DDT per gram of cockroach tissue there was virtually no change and this serves to illustrate the basic difference in susceptibility that exists within this species. In all of the experiments reported in this paper the dosages used are based on the above values.

Adults injected with LD_{50}.—To implement the study of the distribution and metabolism of DDT in the cockroach, adult males and females were compared by injecting them with their respective LD_{50} doses of DDT. The tissues of the injected individuals were assayed at various time intervals after injection ranging from 3 to 96 hours. It was found that no discernible differences occurred in the results during this period of time. Therefore, for purposes of convenience in presentation the results from the various time intervals have been averaged and these values, together with the range for each average value, the percentage recovery of toxicant per tissue, and the total percentage recovery of toxicant are presented in table 3. Each replicate (usually 3) at each time interval is based on the results from 5 females or 10 males. For the sake of simplicity, however, the results are presented on a per cockroach basis. Tests on the nerve cord and brain proved to be completely negative and for that reason these tissues are not included in this or any subsequent table.

Table 3.—DDT and DDE recovered from various tissues of the American cockroach[a] after injection with DDT.

Tissue	No. of Insects	Sex	DDT		DDE		Recovery of Toxicant[b] Per Cent/Tissue	
			Average	Range	Average	Range		
			μg./Roach	μg./Roach	μg./Roach	μg./Roach	Average	Range
Fat body	310	M	0.2	0.0–0.6	0.2	0.0–0.9	10.0	0.0–32.0
	160	F	0.9	0.0–1.8	0.7	0.0–3.1	9.0	0.0–21.0
Gonadial tissue	200	M	0.1	0.0–0.3	0.0	0.0–0.3	2.0	0.0–14.0
	105	F	0.3	0.0–0.7	0.1	0.0–0.8	2.0	0.0– 6.5
Malpighian tubules	200	M	0.1	0.0–0.3	0.1	0.0–0.6	4.0	0.0–20.0
	105	F	0.1	0.0–0.5	0.1	0.0–0.7	1.0	0.0– 6.5
Alimentary canal	210	M	0.7	0.0–1.6	0.1	0.0–0.8	15.0	0.0–32.0
	105	F	2.8	1.1–5.4	0.3	0.0–1.7	16.0	5.5–27.0
Thoracic muscles	210	M	0.5	0.2–0.8	0.1	0.0–0.8	12.0	4.0–30.0
	105	F	2.1	1.0–3.4	0.1	0.0–0.8	11.5	5.0–18.0
Remaining tissues	190	M	1.1	0.3–2.6	0.5	0.0–1.8	32.0	6.0–72.0
	100	F	3.5	0.8–8.7	1.3	0.0–5.7	24.0	4.0–60.0
Outside[c] rinse	340	M	0.5	0.0–1.1	—	—	10.0	0.0–11.0
	185	F	1.2	0.0–5.6	—	—	6.0	0.0–26.0

[a] Adult males injected with 5 and adult females with 20 micrograms.
[b] The total percentage recovery of toxicant averaged 84.5% and ranged from 33.6 to 117.2% in males, while in females it averaged 68.5% and ranged from 38.3 to 83.4%.
[c] The DDT found here represents that which leaked from the needle wound and was obtained by rinsing the insects with ether prior to dissection.

Table 4.—DDT and DDE recovered from the tissues of adult male and female roaches 48 hours after injection with DDT.

Tissue	Dosage	No. of Insects	Sex	DDT		DDE		Recovery of Toxicant[a] Per Cent/Tissue	
				Average	Range	Average	Range		
	μg.			μg./Roach	μg./Roach	μg./Roach	μg./Roach	Average	Range
Fat body	10	15	M	0.3	0.2–0.5	0.0	0.0–0.0	3.3	1.8– 5.2
	10	15	F	0.3	0.2–0.4	0.1	0.0–0.2	2.7	1.8– 4.0
	15	20	M	0.4	0.2–0.6	0.0	0.0–0.0	2.9	1.2– 5.3
	15	20	F	1.0	0.7–1.2	0.2	0.0–0.7	7.5	4.8–12.6
Alimentary canal	10	15	M	2.3	1.5–3.4	0.1	0.0–0.4	23.1	15.2–33.8
	10	15	F	1.4	0.9–2.1	0.4	0.2–0.6	18.0	11.0–23.8
	15	20	M	4.2	3.2–4.9	0.0	0.0–0.0	27.7	21.6–32.8
	15	20	F	2.0	1.3–2.5	0.6	0.0–2.0	17.1	8.6–26.0
Thoracic muscles	10	15	M	0.8	0.3–1.3	0.0	0.0–0.0	8.3	2.8–13.4
	10	15	F	1.7	1.3–2.2	0.1	0.0–0.3	17.7	14.2–24.6
	15	20	M	0.6	0.3–1.0	0.0	0.0–0.0	4.0	1.9– 6.6
	15	20	F	1.5	1.2–2.3	0.0	0.0–0.0	9.8	7.3–15.3
Remaining tissues	10	15	M	2.2	1.5–2.8	0.0	0.0–0.0	22.0	15.2–27.6
	10	15	F	2.1	1.8–2.4	0.0	0.0–0.0	21.1	18.2–23.6
	15	15	M	3.1	3.1–3.2	0.0	0.0–0.0	20.8	20.6–21.3
	15	15	F	3.4	3.1–3.9	0.2	0.0–0.6	24.4	20.6–26.6
Outside rinse	10	15	M	0.4	0.1–0.8	—	—	4.3	0.8– 7.8
	10	15	F	0.1	0.0–0.3	—	—	0.9	0.0– 2.8
	15	20	M	0.7	0.6–0.8	—	—	4.4	4.0– 5.2
	15	20	F	0.0	0.0–0.0	—	—	0.0	0.0– 0.0

[a] The total percentage recovery of toxicant in males averaged 62.1% and ranged from 50.0 to 74.9% at 10 micrograms, and averaged 59.5% with a range of 52.0 to 63.7% at 15 micrograms. In females the corresponding figures are: ave. 61.2%, range 47.6 to 76.9%; ave. 58.2%, range 56.3 to 61.2%

From the information presented in table 3, it appears that DDT injected into the blood stream becomes rather universally distributed in the roach. The distribution does not appear to be equal, however, because the major portion of DDT was recovered from the alimentary canal, the thoracic muscles, the fat body and the remaining tissues in both sexes. Some DDT was found in all of the tissues although the recovery was not always consistent. With respect to the storage of DDT metabolites or possibly the actual metabolism of DDT to DDE it appears that the fat body is the most important tissue in both males and females with the alimentary canal and the remaining tissues also being effective in this respect. Some metabolites occurred in most of the other tissues tested. It should be pointed out, however, that because of the extremely small quantities of DDE recovered in many cases the actual values reported may not be very reliable, but probably do indicate the presence of DDE.

One fact which was shown by these data is that the total percentage recovery of DDT is not good nor is it consistent. While this is somewhat disconcerting, it is not unusual with this type of experiment, In most papers dealing with the detoxification of DDT in insects a similar situation exists. The authors of these papers usually postulate the formation of unknown metabolites of DDT to account for the lost toxicant.

Adults injected with equal amounts.—In the preceding section the roaches were injected with male or female LD_{50} values of DDT which on a dosage-response curve would represent relatively equal dosages. It was also desirable to compare the reactions of the two sexes to a given dose. Accordingly, the results of such experiments are presented in this section In selecting appropriate dosages it was necessary to consider that for purposes of these tests it is best to employ toxicant concentrations which operate in the sublethal range. This somewhat limited the selection because theoretically the LD_{50} for males will kill none of the females and the LD_{50} for females will be lethal to all of the males. Therefore, dosages had to be chosen intermediate to these extremes. Concentrations of 10 and 15 micrograms per cockroach fulfilled the desired requirements. Because the rate and extent of distribution and metabolism of DDT in the tissues does not appear to vary markedly from 3 to 96 hours after injection a single time interval (48 hours) was used for this phase of the study.

The results from these experiments are presented in table 4. In each replicate the tissues of 5 males or 5 females were used. Because of the apparently insignificant role of the gonadial tissue and Malpighian tubules in the disposition of DDT, as indicated in the preceding section, these tissues were not used.

The most striking feature of the data in table 4 is the marked increase in the amount of DDT found in the alimentary canal of the males. This amount appears to be far out of proportion with the increase in dosage of DDT over the preceding data (Table 3). In fact, the amount of DDT present in the alimentary canal of males injected with 15 micrograms is greater than that found in the alimentary canal of females injected with 35 micrograms as will be seen in the following section.

DDT injected into adult females and last-instar nymphs.—The results presented in this section are those from tests on the distribution and metabolism of DDT in two developmental stages of the roach. For this purpose adult females and last-instar male and female nymphs were used. As mentioned earlier, these groups do not

Table 5.—DDT and DDE recovered from various cockroach tissues 48 hours after injection.[a]

Tissue	Sex[b]	DDT		DDE		Recovery of Toxicant[c] Per Cent/Tissue	
		Average	Range	Average	Range		
		μg./Roach	μg./Roach	μg./Roach	μg./Roach	Average	Range
Fat body	A-F	1.7	1.0–2.4	0.4	0.2–0.6	6.0	2.9– 8.6
	N-F	0.6	0.5–1.2	0.5	0.0–1.2	3.4	1.8– 5.7
	N-M	0.3	0.1–0.5	0.5	0.2–1.0	2.5	0.1– 5.0
Alimentary canal	A-F	3.6	2.7–4.4	0.5	0.4–0.5	11.9	9.1–14.2
	N-F	5.0	4.6–5.6	0.2	0.1–0.3	14.6	13.1–16.3
	N-M	5.6	3.7–7.4	0.3	0.2–0.5	16.9	12.0–22.8
Thoracic muscles	A-F	4.4	3.5–5.2	0.2	0.0–0.6	13.5	10.0–15.6
	N-F	4.0	3.7–4.3	0.1	0.1–0.1	11.5	10.5–12.3
	N-M	5.0	3.0–8.4	0.0	0.0–0.0	14.4	8.5–24.9
Remaining tissues	A-F	6.1	4.8–8.2	0.0	0.0–0.0	17.7	13.6–23.4
	N-F	3.6	2.2–5.2	0.0	0.0–0.0	10.4	6.3–14.9
	N-M	2.3	1.4–2.9	0.0	0.0–0.0	6.3	4.0– 8.3
Outside rinse	A-F	1.9	0.4–3.1	—	—	5.5	1.1– 8.9
	N-F	0.3	0.1–0.5	—	—	0.8	0.0– 1.3
	N-M	0.2	0.0–0.5	—	—	0.5	0.0– 1.4

[a] Female adult and male and female last-instar nymphs injected with 35 micrograms of DDT.
[b] Fifteen cockroaches used in each category.
[c] The total percentage recovery of toxicant was as follows: adult female ave.—54.9%, range 47.6 to 64.7%; nymphal female ave.—39.6%; range 33.9 to 49.6%; nymphal male ave.—40.7%; range 28.8 to 47.4%.

differ significantly in their susceptibility to DDT as measured by dosage-mortality data. While it would have been desirable to compare adult males as well, these have been adequately compared with adult females and that is probably sufficient. In any case, adult males subjected to the dosage of DDT used in these tests (35 micrograms per cockroach) would all have been killed.

In each of the replicates of these tests 5 individuals from each of the 3 categories of insects were used. The results are presented in table 5, and as in the preceding section only the more active tissues were tested with the others being left in the carcass. Also, only the 48-hour time interval after injection was used.

The results in table 5, indicate that last-instar nymphs of the two sexes resemble each other more closely than they do adult females with respect to the distribution of DDT. It can be seen that the alimentary canal of the adult female contains less DDT than the corresponding organ in either sex of the nymphs, while the reverse is true of the fat body and the remaining tissues. The greatest amount of metabolism of DDT appears to have occurred in the fat body and the alimentary canal.

Distribution of DDT in the alimentary canal.—The presence of large amounts of DDT in the alimentary canal suggested the importance of localizing the site of absorption. Before this was done, however, it seemed advisable to investigate the possibility of accidental injection of the alimentary canal during administration of the toxicant. This was accomplished by injecting cockroaches with the dye Sudan III. Approximately 50 individuals so treated were dissected and examined under a binocular microscope. In no case was the dye found anywhere except free in the haemocoele or adhering to the various tissues. It was distributed throughout the body but most of it remained in the thorax. These results indicate that the point of injection is well controlled and that any DDT appearing in or on the alimentary canal is the result of absorption from the haemolymph. It was considered likely that most of the toxicant would be found in the fore-gut because of the thoracic site of injection. Accordingly, for these experiments alimentary canals from DDT-treated cockroaches were divided into fore-gut and mid- and hind-gut. The results are presented in table 6 and are based on replicates consisting of 5 males or 5 females sacrificed 48 hours after injection.

From the results presented in table 6, it appears that most of the DDT is present in the fore-gut. It is also evident that DDT is not confined to this part of the alimentary canal. Such information appears to confirm the finding of small amounts of DDT in the posteriorly located tissues such as the gonadial tissue and Malpighian tubules. However, this does not mean that DDT is passed from the alimentary canal. In fact, tests on the fecal material proved to be completely negative up to 96 hours after injection.

Table 6.—Localization of DDT in the alimentary canal of the cockroach 48 hours after injection.[a]

Tissue	DDT in Male' Alimentary Canal		DDT in Female Alimentary Canal	
	Toxicant Present	Content as Per Cent of Total	Toxicant Present	Content as Per Cent of Total
	μg./Roach		μg./Roach	
Fore-gut	2.2	82.7	3.0	81.7
	1.8	69.4	7.3	91.4
	1.2	68.2	4.4	75.9
Mid- and hind-gut	0.5	17.3	0.7	18.3
	0.8	30.6	0.7	8.6
	0.6	31.8	1.4	24.1

[a] Adult males injected with 10 and adult females with 20 micrograms of DDT.

In vitro recovery of DDT and DDE from tissues.—In experiments of the type which have been described in this paper it is obvious that the ability to recover insecticides from the various tissues is important. This is particularly true in view of the poor total recovery of toxicant reported. In an effort to determine how well DDT and DDE can be recovered from cockroach tissues the following experiments were undertaken: known amounts of DDT or DDE were added to the various dissected tissues and the insecticide extracted as soon as possible. These extracts were assayed as previously described. It was found that DDT is recovered in virtually theoretical amounts from all of the tissues. In the case of DDE, however, the recovery was less than theoretical. In spite of this, a satisfactory recovery was achieved in all of the tissues except the fat body and the remaining tissues. With these two tissues the percentage recovery ranged from 20 to 45 per cent. Such a difference in the recovery of DDT and DDE is difficult to explain in view of their chemical similarity.

While these experiments do not prove what can be expected from *in vivo* tests, they do give some indication. Particularly, it is probable that poor *in vitro* recovery means poorer *in vivo* recovery.

DISCUSSION.—It is evident from the results presented in this paper that when DDT is injected into the haemolymph of the American cockroach it is distributed throughout the body. As was previously mentioned, however, this distribution does not appear to be equal. Of the internal tissues and organs containing DDT the thoracic muscles and fore-gut seem to have the most. Upon analysis, this is probably what is to be expected because of the thoracic site of injection and the water insoluble nature of DDT. In fact, when this information is taken into account it is quite remarkable that such a general distribution was achieved. There are probably at least three factors contributing to this widespread distribution of DDT. The most important of these would seem to be the presence of fat in the blood such as fat absorbed from the alimentary canal. It is generally known that DDT is highly fat soluble and it seems plausible to expect any fat contained in the blood to absorb DDT and to carry it away from the site of injection. It also seems plausible to expect that DDT transported in this manner might be lost by the blood fat to fat in other parts of the body by preferential absorption or by some other means. This could be of major importance in the distribution of DDT if the cycle were completed over and over again. Similarly, it is possible that DDT is mechanically distributed by becoming suspended in the blood as it moves about among the various parts of the body. The open circulatory system of the cockroach would certainly be an aid to such movement. In addition it is conceivable that an equilibrium is established between the non-lipoid portion of the blood and DDT. While such a factor is probably of minor or perhaps only theoretical significance in the distribution of DDT, is should not be overlooked.

When the metabolism of DDT to DDE is considered it appears that in the cockroach the fat body, the alimentary canal, and the remaining tissues are of the greatest significance as sites of metabolism, although some DDE was found in most of the tissues tested. It should be pointed out, however, that merely finding DDE in a tissue does not prove that it was formed there. In spite of this, it does seem likely that metabolism of DDT is occurring in the three tissues mentioned above because of the fairly large amounts of DDE found in them. This is particularly true when it is remembered that the *in vitro* recovery of DDE from the fat body and the remaining tissues was very poor. This could mean that these two tissues are of considerably greater importance in this respect than they appear to be. In other instances, however, it is conceivable that DDE is formed in one tissue and transported by the blood to another. This could have happened with the gonadial tissue, for example, where only small amounts of DDE were recovered. It is also possible that DDT is being metabolized to other products which may or may not respond to the Schechter-Haller procedure, and whose sites of formation may or may not correspond to those for DDE.

There are two features about the distribution and sites of metabolism of DDT in the cockroach which might be correlated with the differential susceptibility of the sexes and developmental stages. The more important of these would seem to be the apparent significance of the fat body in the metabolism of DDT to DDE. This is of importance because the adult females and last-instar male and female nymphs all have considerably larger fat bodies than the adult males. It is obvious that this could be of major importance in explaining the differential susceptibility mentioned above, if the metabolism of DDT to DDE plays a role in determining susceptibility. The second feature which is possibly of importance in this respect is the larger amount of DDT found in the alimentary canal of adult males injected with a dosage of 15 micrograms. As was pointed out, this amount is greater than that found in the alimentary canal of adult females injected with 35 micrograms. The interesting feature here is that a substantially smaller percentage of the males would survive at the 15 microgram dosage than would females at the 35 microgram dosage. It is possible that one of the reasons why these males were able to survive is that they retained large quantities of DDT in or on their alimentary canals. This amount, which averaged better than 25 per cent of the total dose, probably does them little harm and could be the difference between survival and death. If such a factor is of importance it would apparently have to be effective in nymphs and adult females, as well as in adult males, when they are treated at higher dosages.

SUMMARY.—Studies were made on the distribution and sites of metabolism of DDT injected into the blood stream of the American cockroach. It was found that DDT is present in the alimentary canal, gonadial tissues, fat body, Malpighian tubules, thoracic muscles, and remaining tissues of adult males and females injected with various dosages of DDT. In both sexes the largest amounts were found in the alimentary canal, thoracic muscles, fat body, and remaining tissues. A similar widespread distribution of DDT was found in last-instar nymphal males and females injected with this insecticide.

Metabolism of DDT to DDE apparently takes place in all of the tissues mentioned, although in some to a rather limited extent. In adult males and females DDE was found in the greatest quantities in the fat body, alimentary canal, and remaining tissues.

The possible significance of certain features of this work is discussed in relation to the differential susceptibility to DDT exhibited by the sexes and developmental stages of the cockroach. It was concluded that the fat body probably contributes more to bringing about these differences than the data indicate.

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House Fly Egg-Measuring Techniques[1]

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In rearing insects for the bioassay of insecticides, it is desirable to produce cultures having a high degree of uniformity. When the Pesticide Testing Laboratory was recently organized, it was decided to rear the house fly, *Musca domestica* L., according to the Peet-Grady Method (Anonymous 1955) with one or two slight modifications. In developing the art of rearing this species, techniques for measuring eggs were investigated.

A review of literature revealed that measuring techniques for house-fly eggs for mass rearing were invariably volumetric, depending on calibrated graduates, centrifuge tubes, or pipettes. The Peet-Grady Method (Anonymous 1955) mentioned the use of calibrated pits or cells, and this method has been seen in certain laboratories, though it was not described in the literature reviewed. Usually little or no detail on the standardization of the egg-measuring technique was reported. Several authors (Anonymous 1955, Campbell & Sullivan 1938, Eagleson 1943, Fisher 1952, West 1951, Woodbury 1943) mentioned washing the eggs in water and allowing them to settle in a graduated measure. Others (Allen *et al.* 1943, Bickoff 1943, Cox 1944, Peterson 1953) would lead one to the conclusion that they also had employed settling in water. Wilkes *et al.* (1948) used a tube capped with screening but specified that "at least a 2-in. head of water was maintained in the tube above the eggs." Some authors (Harrison 1949, McKenzie & Hoskins) mentioned the use of pipettes but gave no details of their techniques. Fisher & Morrison (1949) provided an adequate description and emphasized that the eggs were settled by tapping. They stated, "The numbers depend on the consolidation." Clnsidering the importance of this truth, it is surprising that other writers have not also referred to it.

The need for standardizing the consolidation of eggs becomes evident if one studies the different egg counts reported. Reducing all counts to a basis of eggs per 0.1 ml., we find records of 500 (Cox 1944, West 1951, Woodbury 1943); 600 (Fisher & Morrison 1949) 700 (Anony-

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mous 1955, Basden 1947, McKenzie & Hoskins 1954,[2] Peterson 1953); and 750 (Smith & Harrison 1951) eggs.

METHODS AND MATERIALS.—In this laboratory, food dishes in the breeding cages are replaced with oviposition dishes at 9 a.m., and a sufficient supply of eggs is normally deposited within 5 hours. The oviposition pad is cellucotton soaked in hot skim milk, which is better than cold milk, and pressed with a spatula until compact and without surplus moisture.

Eggs are transferred by spatula from the oviposition dish to a 15-ml. centrifuge tube containing tap water at room temperature (Fig. 1A). This tube is shaken to break up egg clumps and wash the eggs. Floating eggs and surplus water are removed by means of a syringe. Addition of clear water (Fig. 1B) and shaking and removal of floating eggs are repeated a second and a third time.

For counting purposes, the eggs were placed in parallel lines on green blotting paper by means of a syringe.

THE GRADUATED CENTRIFUGE TUBE.—The graduated centrifuge tube is used in some laboratories (Campbell & Sullivan 1938, Peterson 1953). It has a diameter large enough for easy manipulation, and the eggs settle in about 1 minute to give a flat surface, easy to measure against the graduations. One disadvantage is that the expanding diameter at the bottom permits a greater percentage error with increased volume of eggs. Each tube must be individually calibrated.

In these trials only one centrifuge tube was used, thus overcoming the problem of calibration, and all measuring was done by one operator. All eggs were new-laid, and each measure was settled for a sufficiently long period. Twenty counts, of 0.4 ml. each, gave an average of 2750 eggs, with a standard deviation per count of ±188 eggs.

THE GRADUATED PIPETTE TUBE.—Portions of 1-, 5-, and 10-ml. pipette tubes were cut and 54-mesh bolting silk was glued to one end of each tube with De Khotinsky cement. The 1-ml. pipette sections proved difficult to handle since the eggs packed too tightly and prevented proper drainage. It was almost impossible to add or remove eggs with a syringe because of the small inside diameter of the tube. The 10-ml. pipette was too large and the curvature of the upper surface of the egg mass made accurate readings difficult. However, 5-ml. pipette tubes, graduated in tenths, were suitable and were used in this investigation.

After the eggs were thoroughly washed, they were

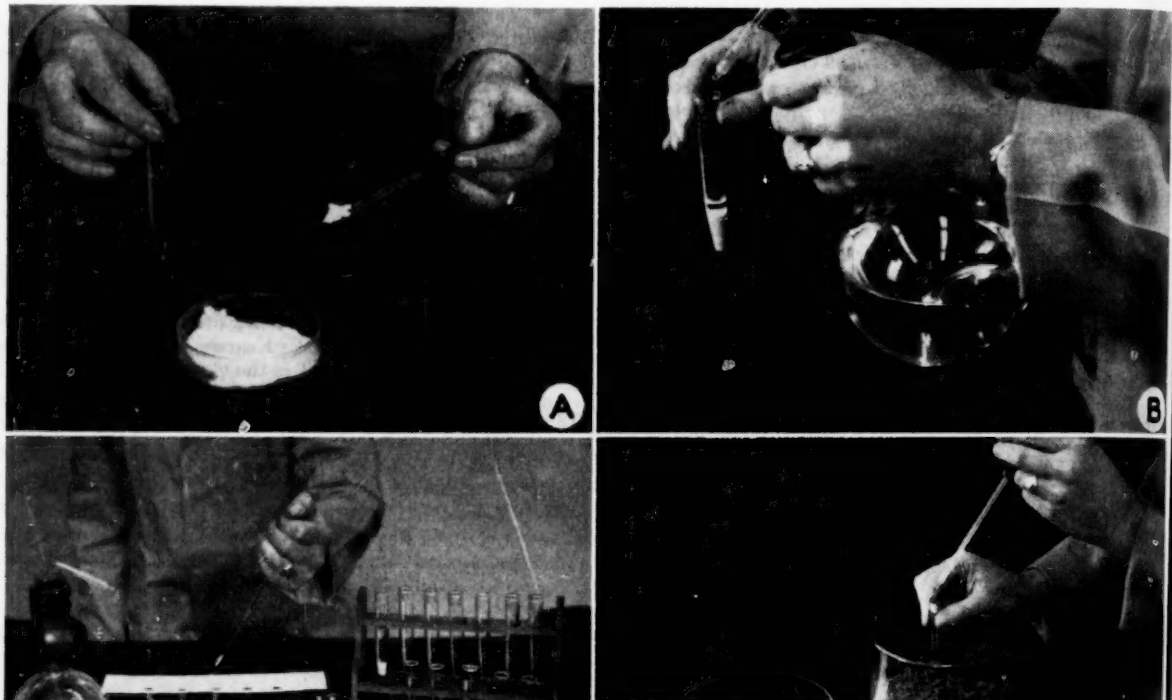

FIG. 1.—Egg measuring and seeding. A. Transferring eggs from oviposition dish to centrifuge tube; B. Washing eggs; C. Transferring washed eggs from centrifuge tube to pipette tube; D. Washing measured volume of eggs into larval medium.

[2] The original publication by McKenzie and Hoskins (1954) stated that "about 2,100 eggs, corresponding to 1.3 ml. volume in a marked pipette, were withdrawn." This was a printing error and should have read "0.3 ml. volume.

transferred to the measuring pipette by means of a syringe (Fig. 1C). The measuring tube stood on a wet cellulose sponge 4 cm. thick, which rested in about 1 cm. of water. The tube was steadied by a cardboard support. Twenty counts, of 0.3 ml. each, gave an average of 2687 eggs, with a standard deviation per count of ±83 eggs.

To achieve such a degree of accuracy, the consolidation of the eggs must be standardized. Several techniques were investigated. The pipette was almost submerged in water, and eggs were added and allowed to settle. If agitated after first settling, they almost always resettled to a different mark, though sometimes the latter could be reproduced following further agitation. Syringing clear water into the pipette consolidated the eggs satisfactorily provided a sufficient quantity of water was used, but the technique was awkward. Exact reproduction of measurement of eggs could always be obtained when the screened end of the tube rested upon a wet cellulose sponge where the wetness was standardized by setting the sponge in free water in a dish. A given volume of eggs, no matter how it was agitated, would always return to the same mark when drained on the sponge.

The pipette method is slow at first but becomes rapid with practice. The tubes should be no longer than 6 cm. since eggs sometimes become stuck in longer lengths. The eggs can be transferred to the rearing medium by inverting the tube and syringing with water (Fig. 1D).

THE PIT METHOD.—Some laboratories favour the use of metal blocks with pits of appropriate size drilled in them. Each pit must, of course, be individually calibrated. An aluminum bar with a single pit was used in these tests. Washed eggs were poured onto bolting silk on a wet cellulose sponge. After draining, they were put into the pit with a spatula, and the top of the bar was scraped clean of free eggs. Eleven counts gave an average of 1265 eggs, with a standard deviation per count of ±366 eggs. No attempt was made to refine this technique further.

REARING TESTS.—By use of the F test, the errors of measurement were shown to differ significantly between methods. However, with biological material, there is no virtue in attempting to refine one's technique beyond the limits imposed by the natural variability of the subject species. Accordingly, flies were reared from eggs measured by centrifuge and pipette methods (the pit method was not tested) and numbers of pupae per jar were recorded. Analyses of variance showed that there was no significant difference in puparial yield between days for either method. The standard deviation of puparial yield did not differ significantly between methods, as indicated by the F test.

DISCUSSION.—Since there was no significant difference in the variation of puparial yields obtained by the two methods of measuring eggs, the centrifuge-tube method is preferable for mass rearing because of ease of manipulation. The graduated pipette tube is more accurate in the measurement of the eggs and might prove to be of value for special research where similar refinement of techniques can be introduced in all phases of the rearing process.

ACKNOWLEDGMENTS.—Our thanks are due to Dr. R. W. Fisher for first showing us the pipette tube closed with bolting silk. We are also grateful to scientists in certain industrial laboratories for their hospitality and willingness to discuss techniques with us. Mr. J. D. Gates assisted with the egg counts and Mr. E. K. Raynor was in charge of rearing work.

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[3] R. W. Fisher.

Some Factors Affecting the Susceptibility of the Armyworm to Virus Infections[1]

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In the spring of 1954, an outbreak of armyworms occurred on the island of Hawaii and destroyed the forage on thousands of acres of range land (Davis 1955). The cosmopolitan armyworm, *Pseudaletia unipuncta* (Haw.),[2] was one of several armyworm species that was causing serious destruction to range grasses. On one of the cattle ranches located at Kohala, Hawaii, a localized population of this armyworm was almost exterminated by a virus epizootic. Many armyworms died in a "wilted" condition, suspended from the tops of grass shoots. Most of the living specimens collected from this area died from apparent polyhedrosis, but one larva died with the typical external symptoms of granulosis and its tissues contained many granules[3] (Tanada 1955). This is the first record of a granulosis virus infecting *P. unipuncta*. A polyhedrosis virus was reported in this species by Chapman & Glaser (1915) and Steinhaus (1951). Another virus, *Morator nudus* Wasser, a form without inclusion body, was described from *P. unipuncta* by Wasser (1952).

Although *P. unipuncta* appeared to be highly susceptible to the polyhedrosis virus in the field, it was relatively resistant under laboratory conditions. In three trials, in which 20, 28, and 15 third- and fourth-instar larvae were fed a heavy suspension of the virus, 45, 25, and 20 per cent, respectively, of the larvae died from polyhedrosis. Comparable numbers of control larvae in the three trials did not develop any virus infection.

Third- and fourth-instar larvae of *P. unipuncta* were also partially resistant to the granulosis virus under laboratory conditions. In two trials in which 30 and 20 third- and fourth-instar larvae were fed the virus, 43 and 35 per cent, respectively, of the larvae died from granulosis.

Inasmuch as it was rather noteworthy to find such marked differences in the susceptibility of *P. unipuncta* larvae under field conditions as compared to those of the laboratory, some of the factors that might affect the susceptibility of *P. unipuncta* to the two viruses were investigated. The following factors were considered: (1) age of the larvae, (2) multiple virus infection, (3) dosage of the virus, and (4) different types of food.

MATERIALS AND METHODS.—The experimental stock of *Pseudaletia unipuncta* was reared from apparently healthy larvae which were collected a short distance from the infected population. All tests were conducted with offspring of this initial stock.

The adults were held in widemouthed gallon jars and were fed daily with sugar water placed in a Syracuse dish filled with cotton. Crumpled pieces of paper towelling were placed in the jars to serve as oviposition sites. The paper and eggs were removed daily and replaced by fresh towelling. Most of the eggs were kept at room temperature and allowed to hatch, but some were stored in a refrigerator at 4° to 6° C. Under this refrigeration they remained viable for 2 to 3 weeks.

Larvae were fed the tender leaves of young shoots of Napier grass, *Pennisetum purpureum* Schumach. The food bouquets were prepared by inserting the ends of grass leaves wrapped in a small amount of cotton into shell vials containing tap water, which kept the leaves turgid for 2 to 3 days.

Larvae were infected by feeding them grass leaves which had been dipped in a heavy suspension of virus. The control larvae were fed leaves dipped in distilled water. Each larva was reared separately in a sterile ½-pint cardboard container covered with a half of a sterile petri dish.

The body contents of every dead larva and pupa were examined under the microscope. Susceptibility determinations were based on mortality from virus infection, which in turn was diagnosed by the presence of the characteristic inclusion bodies observed under the light microscope. A larva that survived to adulthood, after exposure to the virus, was considered as being resistant. Of course, larvae that died from causes other than from a frank virus infection may or may not have been resistant to virus infection.

AGE OF LARVAE.—In the investigation of the relationship of different larval ages to susceptibility to the viruses, the larvae were usually tested 1 to 2 days after molting into a given instar. The treatments with polyhedrosis virus and with granulosis virus were conducted simultaneously in most tests, but in several trials they were tested separately. An equivalent number of control larvae were always maintained during the tests.

The virus inoculum fed to first-instar larvae was permitted to dry overnight on Napier grass in order to destroy most of the nonspore-forming bacteria present in the inoculum. Between 5 and 10 first-instar larvae were fed such treated grass for 2 days before they were placed singly into separate containers.

Four trials were conducted with first-instar larvae using a total of 88 larvae exposed to polyhedrosis, 65 to granulosis, and 72 which served as controls. Mortality caused by polyhedrosis ranged from 69.6 to 95.8 per cent (average, 85.8 per cent), and by granulosis 52.6 to 100 per cent (average, 80.8 per cent).

Two trials were conducted with second-instar larvae. A total of 43 larvae were tested with polyhedrosis virus, 40 with granulosis virus, and 38 served as controls. The average mortality from polyhedrosis was 78.1 per cent; that from granulosis was 92.7 per cent which is higher than that of the first-instar larvae. The apparent lower percentage mortality from granulosis in the first-instar larvae may have been caused by the rather high mortality from other causes, whereas an equivalent mortality might otherwise have resulted from virus infection.

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Table 1.—**Susceptibility of the six larval instars of *Pseudaletia unipuncta* to the polyhedrosis and the granulosis viruses.**

		Polyhedrosis Virus				Granulosis Virus				Control (Virus Absent)			
			Per Cent				Per Cent				Per Cent		
Instars	Trials	Number Treated	Dead from Virus	Dead from Others	Adults	Number Treated	Dead from Virus	Dead from Others	Adults	Number Treated	Dead from Virus	Dead from Others	Adults
I	A	16	93.8	0	6.3	6	100.0	0	0	15	0	46.7	53.3
	B	25	84.0	16.0	0	25	84.0	16.0	0	20	0	25.0	75.0
	C	24	95.8	4.2	0	19	52.6	42.1	5.3	15	6.7	46.7	46.7
	D	23	69.6	26.1	4.4	15	86.7	13.3	0	22	4.6	36.4	59.1
	Total or Average	88	85.8	11.6	2.7	65	80.8	17.9	1.4	72	2.8	38.7	58.5
II	A	18	72.2	11.1	16.7	15	93.3	0	6.7	14	0	0	100.0
	B	25	84.0	0	16.0	25	92.0	4.0	4.0	24	0	37.5	62.5
	Total or Average	43	78.1	5.6	16.4	40	92.7	2.0	5.4	38	0	18.8	81.3
III	A	20	45.0	10.0	45.0	—	—	—	—	11	0	36.4	63.6
	B	28	25.0	28.6	46.4	—	—	—	—	17	0	58.8	41.2
	C	25	36.0	12.0	52.0	24	79.2	8.3	12.5	25	0	36.0	64.0
	D	20	20.0	40.0	40.0	20	85.0	5.0	10.0	20	0	25.0	75.0
	E	—	—	—	—	20	35.0	15.0	50.0	20	0	35.0	65.0
	Total or Average	93	31.5	22.7	45.9	64	66.4	9.4	24.2	93	0	38.2	61.8
IV	A	15	20.0	33.3	46.7	—	—	—	—	15	0	66.7	33.3
	B	15	13.3	46.7	40.0	14	64.3	14.2	21.4	14	0	28.6	71.4
	C	10	40.0	50.0	10.0	10	60.0	20.0	20.0	10	0	30.0	70.0
	D	10	30.0	40.0	30.0	10	50.0	30.0	20.0	10	0	10.0	90.0
	Total or Average	50	25.8	42.5	31.7	34	58.1	21.4	20.5	49	0	33.8	66.2
V	A	9	11.1	11.1	77.8	9	11.1	11.1	77.8	9	0	55.6	44.4
	B	18	22.2	50.0	27.8	16	31.3	0	68.8	18	0	33.3	66.7
	C	20	15.0	5.0	80.0	20	25.0	10.0	65.0	20	0	10.0	90.0
	Total or Average	47	16.1	22.0	61.9	45	22.5	7.0	70.5	47	0	33.0	67.0
VI	A	15	6.7	26.7	66.7	15	20.0	20.0	60.0	15	6.7	13.3	80.0
	B	15	0	33.3	66.7	15	20.0	26.7	53.3	15	0	13.3	86.7
	Total or Average	30	3.4	30.0	66.7	30	20.0	23.4	56.7	30	3.4	13.3	83.4

Third-instar larvae were tested in four trials with polyhedrosis virus and in three trials with granulosis virus. Mortality resulting from polyhedrosis ranged from 20 to 45 per cent, with an average of 31.5 per cent; that from granulosis ranged from 35 to 85 per cent, with an average of 66.4 per cent.

A total of 50 fourth-instar larvae were treated with polyhedrosis virus in four trials, and a total of 34 fourth-instar larvae were treated with granulosis virus in three trials. Mortality was 13.3 to 40 per cent from polyhedrosis (average, 25.8 per cent), 50 to 64.3 per cent from granulosis (average, 58.1 per cent).

Tests with fifth-instar larvae were conducted in three trials with a total of 47 larvae treated with polyhedrosis virus and 45 larvae with granulosis virus. The mortality ranged from 11.1 to 22.2 per cent for polyhedrosis (average, 16.1 per cent) and from 11.1 to 31.3 per cent for granulosis (average, 22.5 per cent).

Sixth-instar larvae were tested in two trials, each of which contained 15 larvae. An average of 3.4 per cent died from polyhedrosis and 23.4 per cent from granulosis.

In the entire study only three larvae of the controls died from virus infection. Two died in trials with first-instar larvae and one in a trial with sixth-instar larvae. Most of the deaths resulting from causes other than virus infection occurred during the prepupal and pupal stages. Such deaths occurred in the majority of the control treatments.

The results of the above study are presented in table 1 and figure 1. They indicate that the first two instars were highly susceptible to both the polyhedrosis and the granulosis viruses. As the larvae became older, they were increasingly resistant to the two viruses. The larvae appeared to be much more susceptible to granulosis than to polyhedrosis virus. As regards the polyhedrosis, each instar showed some difference in the period of lethal

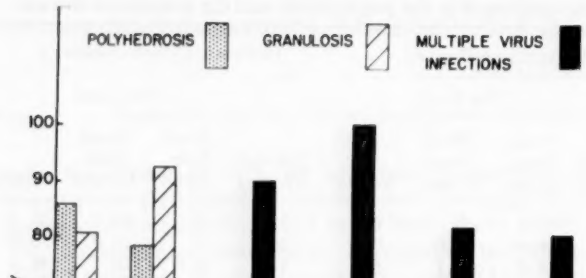

FIG. 1.—Susceptibility of the six larval instars of *Pseudaletia unipuncta* to polyhedrosis, granulosis, and multiple virus infections. The first and second instar larvae were not tested with a mixture of the two viruses.

infection, i.e., the period beginning with the larva feeding on the virus until its death from a frank virus infection. Most of the first-instar larvae died in 4 to 6 days after feeding on the virus, the second instar in 5 to 7 days, the third instar in 7 to 10 days, and the fourth instar from 9 to 11 days. The fifth- and sixth-instar larvae were so highly resistant to infection that their numbers dying from virus infection were insufficient for the adequate determination of the period of lethal infection.

The period of lethal infection for first-instar larvae infected with granulosis virus was from 3 to 34 days. Most of the second-, third-, and fourth-instar larvae died in 14 to 24 days after feeding on the virus. The period of lethal infection for these three instars did not differ as it did with polyhedrosis. The numbers of larvae of the fifth and sixth instars which died from granulosis were insufficient for the adequate determination of their period of lethal infection.

MULTIPLE VIRUS INFECTION.—The above study indicated that the resistance to virus infections increased in the third- to sixth-instar larvae. Subsequent studies were conducted with these more resistant stages of the armyworm.

Inasmuch as the polyhedrosis and granulosis viruses were isolated from armyworms collected from the same infected area, the question arose: Was the armyworm susceptible to a multiple virus infection? The susceptibility of the older larvae to a mixture of polyhedrosis and granulosis viruses was compared with their susceptibility to the individual virus.

The inoculum of polyhedrosis virus and that of granulosis virus were prepared as described previously. The mixed virus inoculum was prepared by combining equal volumes of the two virus inocula. The resultant mixture of the two viruses thus contained roughly half the amount of polyhedrosis virus and of granulosis virus found in the original inocula.

Each trial consisted of the following treatments: (1) larvae fed polyhedrosis virus, (2) larvae fed granulosis virus, (3) larvae fed a mixture of the two viruses, and (4) control larvae which were fed no virus. The results are presented in table 2 and incorporated into figure 1 for comparison with the results of the previous study.

Only one trial was conducted with third-instar larvae. Each treatment had 20 larvae and the mortality percent-

Table 2.—Effect of a mixture of polyhedrosis and granulosis viruses on the susceptibility of third- to sixth-instar larvae of *Pseudaletia unipuncta* to virus infections.

		Polyhedrosis Virus		Granulosis Virus		Mixture of Viruses		Control (Virus Absent)	
Instars	Trials	Number Tested	Per Cent Dead from Virus	Number Tested	Per Cent Dead from Virus	Number Tested	Per Cent Dead from Virus	Number Tested	Per Cent Dead from Virus
III	A	20	20.0	20	85.0	20	90.0	20	0
IV	A	10	40.0	10	60.0	10	100.0	10	0
	B	10	30.0	10	50.0	10	100.0	10	0
	Total or average	20	35.0	20	55.0	20	100.0	20	0
V	A	9	11.1	9	11.1	9	66.7	9	0
	B	18	22.2	16	31.3	17	88.8	18	0
	C	20	15.0	20	25.0	20	90.0	20	0
	Total or average	47	16.1	45	22.5	46	81.8	47	0
VI	A	15	6.7	15	20.0	15	80.0	15	6.7
	B	15	0	15	20.0	15	80.0	15	0
	Total or average	30	3.4	30	20.0	30	80.0	30	3.4

ages from virus infections were 20 for polyhedrosis, 85 for granulosis, and 90 for the mixture of viruses. There were two trials conducted with fourth-instar larvae; both trials had 10 larvae per treatment. The average mortality percentages were 35 for polyhedrosis, 55 for granulosis, and 100 for the mixture of viruses. Fifth-instar larvae were tested in three trials with a total of 47 larvae treated with polyhedrosis virus, 45 with granulosis virus, 46 with mixture of viruses, and 47 larvae used as controls. An average of 16.1 per cent died from polyhedrosis, 22.5 per cent from granulosis, and 81.8 per cent from mixture of viruses. Two trials were conducted with sixth-instar larvae; both trials had 15 larvae per treatment. The average mortality percentages were 3.4 for polyhedrosis, 20.0 for granulosis, and 80.0 for the mixture of viruses.

The above results confirmed the previous observation that the armyworm, as it became older, was increasingly resistant to both the polyhedrosis and the granulosis viruses. However, the older larvae were highly susceptible to a mixture of the two viruses. The fifth and sixth larval instars appeared slightly less susceptible to the mixture of the viruses than the third and fourth instars. Larvae when fed a mixture of the two viruses exhibited external symptoms typical of polyhedrosis, i.e., the characteristic symptoms of "wilt." Their infected tissues, however, occasionally contained both polyhedra and granules, which indicated that a multiple virus infection had occurred. The period of lethal infection with multiple virus was approximately 6 to 8 days for the third larval instar, 6 to 10 days for the fourth instar, 7 to 11 days for the fifth instar, and 8 to 12 days for the sixth instar.

DOSAGE OF VIRUS INOCULUM.—In the previous studies, no attempt was made to determine the actual concentration of the virus in the inoculum. In every instance, however, the inoculum was a heavy virus suspension, and it was apparent that increasing the virus concentration *per se* would not significantly increase the susceptibility of the armyworm. Therefore, instead of varying the concentration of the virus, the following study was conducted to determine whether continuous feeding of the virus over a period of time would result in increased infection. The test larvae consisted of two groups: (1) those reared on Napier grass, and (2) those reared on Kikuyu grass, *Pennisetum clandestinum* Hochst. Each group was treated with single or repeated doses of the polyhedrosis virus, the granulosis virus, and a mixture of the viruses. The control larvae were fed untreated grass. There were two trials, each of which had 10 larvae per treatment. In the first trial, most of the larvae were in their third instar, with a few in their early fourth instar; the second trail was conducted with fourth-instar larvae.

Larvae that received repeated doses of virus were exposed continuously to virus-treated leaves for six changes of food which extended over a period of 12 days for the first trial and 11 days for the second trial. Different virus inocula were used for the two trials, but in each trial the same inoculum was used throughout. There was apparently little or no difference in susceptibility of the larvae when fed the viruses only once, as compared to continuous feeding for 11 to 12 days (Table 3). The possible exception occurred in the group of larvae reared on Kikuyu grass and fed the polyhedrosis virus, in which case continuous feeding resulted in 80 per cent average mortality as compared to 30 per cent for a single feeding. This exception needs to be confirmed by further trials. As in the previous study, the mixture of the two viruses caused nearly 100 per cent mortality for larvae reared on Napier or Kikuyu grass. The two different types of food, Napier and Kikuyu grasses, appeared to have no marked effect on the susceptibility of the larvae.

Table 3.—Effect of single and repeated feeding of the polyhedrosis and granulosis viruses on the susceptibility of *Pseudaletia unipuncta* to virus infections.[a]

Virus Treatments	Number of Times Fed Virus	Larvae Fed Napier Grass			Larvae Fed Kikuyu Grass		
		Per Cent Dead from Virus	Per Cent Dead from Others'	Per Cent Adults	Per Cent Dead from Virus	Per Cent Dead from Others	Per Cent Adults
Polyhedrosis	Single	40	25	35	30	10	60
	Repeated	50	20	30	80	5	15
Granulosis	Single	65	10	25	60	5	35
	Repeated	35	25	40	70	0	30
Mixture	Single	100	0	0	100	0	0
	Repeated	100	0	0	90	10	0
Control	—	0	20	80	0	5	95

[a] There were two groups of larvae, one of which was reared on Napier grass and the other on Kikuyu grass. Average of two trials, each of which had 10 larvae per treatment.

DIFFERENT TYPES OF FOOD.—Inasmuch as the armyworms used in the present investigation were reared on Napier grass rather than on Kikuyu grass, which was the food of the population destroyed by the virus epizootic, it was possible that the food might have been responsible for the greater resistance shown by the laboratory specimens. The above study has indicated, however, that armyworms which had been reared continuously either on Napier grass or on Kikuyu grass showed little or no difference in their susceptibility to virus diseases.

Another trial was conducted to determine whether a change in food from Napier to Kikuyu grass would affect larval susceptibility. Fourth-instar larvae reared on Napier grass were divided into two groups: one of these was retained on Napier grass and the other was transferred to Kikuyu grass. Each group was treated with polyhedrosis and granulosis viruses. There were about 15 larvae per treatment. The transfer of larvae from Napier to Kikuyu grass resulted in little change, if any, in susceptibility (Table 4). The reciprocal test with larvae reared on Kikuyu grass and transferred to Napier grass was not made.

A similar study was made with Napier grass and corn, *Zea mays* Linn. In the first trial, larvae that had been reared on Napier grass were divided into two groups: one group was maintained on Napier grass, and the other was transferred to corn. Each group was further subdivided into the following treatments: (1) larvae fed polyhedrosis virus, (2) larvae fed granulosis virus, (3) larvae fed a mixture of viruses, and (4) untreated control larvae. Each treatment had 15 larvae, most of which were in their fourth instar, with a few in the late third instar. The results showed little if any difference in the susceptibility of the larvae which were transferred from Napier grass to corn (Table 4).

A reciprocal study was conducted with larvae reared on

Table 4.—Susceptibility of *Pseudaletia unipuncta* to virus infections when transferred from a first host to a second host.

Virus Treatments	First Host				Second Host			
	Number				Number			
	Larvae Tested	Dead from Virus	Dead from Others	Adults	Larvae Tested	Dead from Virus	Dead from Others	Adults
	Napier Grass				*Kikuyu Grass*			
Polyhedrosis	15	2	7	6	15	6	1	8
Granulosis	14	9	2	3	15	3	0	12
Control	14	0	4	10	15	0	3	12
	Napier Grass				*Corn*			
Polyhedrosis	15	1	3	11	15	3	3	9
Granulosis	15	10	1	4	15	8	3	4
Mixture	15	13	0	2	15	14	1	0
Control	15	0	3	12	15	0	2	13
	Corn				*Napier Grass*			
Polyhedrosis	10	2	0	8	10	3	4	3
Granulosis	10	2	0	8	10	5	3	2
Mixture	10	10	0	0	10	10	0	0
Control	10	0	2	8	10	0	1	9

corn and transferred to Napier grass. There were 10 fourth-instar larvae per treatment. The results again indicated that a change of food from corn to Napier grass did not markedly affect susceptibility of *P. unipuncta* to virus infections (Table 4). The high larval susceptibility to a mixture of the two viruses in the above two trials confirmed the results obtained in the previous study.

DISCUSSION.—Subsequent to the first record of Chapman and Glaser in 1915 that a polyhedrosis virus disease occurred in *Pseudaletia unipuncta* several reports have appeared on the polyhedrosis of this species, but none of them has made any reference to the susceptibility of the armyworm to virus infections. The present study was stimulated by field observations of a virus epizootic of *P. unipuncta* that indicated a rather high susceptibility of this species to virus infections, whereas under laboratory conditions the armyworm was relatively resistant to infection by two viruses—a polyhedrosis and a granulosis virus isolated from field-collected armyworms.

The differential susceptibility of larvae of different ages to virus infections is generally known (Bergold 1943, Steinhaus 1949). According to Glaser (1928), all larval instars of the silkworm, *Bombyx mori* (Linn.), were susceptible to polyhedrosis virus, but older larvae were more easily infected than the young. Bergold (1943), however, found that the larvae of *Bombyx mori* (Linn.), *Porthetria dispar* (Linn.), and *Lymantria monacha* Linn. did not all die when infected with virus in their more advanced stages of growth. He further reported in 1951 that the polyhedrosis virus of the spruce budworm, *Choristoneura fumiferana* (Clem.), frequently did not kill the larvae when it was fed to them 10 days before pupation. Smith *et al.* (1953) claimed that the privet hawk moth, *Sphinx ligustri* (L.), was most susceptible to polyhedrosis virus during its early larval stage. Bird (1953) and Bird & Whalen (1953) observed that the prepupal stages of the European spruce sawfly, *Diprion hercyniae* (Htg.), and the European pine sawfly, *Neodiprion sertifer* (Geoffroy), were immune to infection by polyhedrosis viruses and that the immunity was associated with the "embryonic cells" lining the midgut during the prepupal stage. Older larvae of the imported cabbageworm, *Pieris rapae* (L.), appeared much more resistant than the young to the granulosis virus, *Bergoldia virulenta* Tanada (Tanada 1953). Lower (1954) also noted that larvae of *Persectania ewingii* Westw. infected towards the end of their larval period were more resistant to a granulosis virus.

The resistance of the armyworm to the polyhedrosis and the granulosis viruses increased directly with the age of the larvae. First- and second-instar larvae were highly susceptible to both viruses (over 78 per cent mortality), whereas the fifth and sixth instars were highly resistant (less than 23 per cent mortality). Such increase in resistance associated with the growth of the larva is regarded by some authors as a "maturation immunity."

The different instars of *P. unipuncta* appeared more susceptible to the granulosis virus than to the polyhedrosis virus.

The dosage of virus inoculum is obviously important in determining whether infection will take place in susceptible hosts. Most insect viruses reported so far are highly virulent, and a relatively minute dose is sufficient to cause lethal infection. In the present study, concentrated suspensions of either the polyhedrosis or granulosis virus failed to cause lethal infection in the majority of the older *P. unipuncta* larvae. Moreover, continuous exposure of the larvae for 11 to 12 days to food dipped in either of these two virus inocula did not markedly increase larval susceptibility.

Different types of food and the sudden change from one type of food to another are known to affect the susceptibility of insects to virus infections (Vago 1951). In experiments with *P. unipuncta*, however, no marked increase in susceptibility was noted when the armyworm was raised either on Napier grass, Kikuyu grass or corn. Neither did changes in food from Napier to Kikuyu grass or corn, and from corn to Napier grass significantly increase the susceptibility of the armyworm.

In the present study, the most important factor that affected the susceptibility of *P. unipuncta* to virus infections was the apparent synergistic relationship which resulted in increased virulence of the two viruses when they were fed to the armyworm as a mixed inoculum. Larvae from the third to sixth instars, which are relatively resistant to the polyhedrosis and granulosis viruses, were highly susceptible when fed a mixture of the two. Larvae infected by a mixture of the two viruses generally died with symptoms of polyhedrosis, and their tissues contained mainly polyhedral inclusion bodies; but some of the infected insects had both polyhedra and granules in their tissues indicating a multiple virus infection. The preponderance of polyhedra over granules in the multiple infection appeared to be correlated with the shorter incubation period of polyhedrosis. Thus during the brief periof of lethal infection from the two viruses there was opportunity for more polyhedra than granules to be produced. Occasionally a long period of lethal infection occurred, and the number of granules was as great or greater than that of polyhedra.

Mutualistic relationship of two microorganisms has been used as a method to increase the virulence of pathogens (Steinhaus 1949). Such mutualistic relationship is known for plant and certain animal viruses, but this is apparently the first reported example found in insect viruses. The apparent lower infectivity of the polyhedrosis virus indicates that it may be more dependent on the mutualistic association than the granulosis virus in order to cause infection. This problem is now

being investigated.

Although the mixture of polyhedrosis and granulosis viruses was highly virulent to *P. unipuncta* it was not infectious when fed to *Pieris rapae* and *Spodoptera mauritia* (Boisduval). Definite conclusions cannot be drawn with cross-infectivity tests conducted with only two insect species, but the above tests apparently indicate that the increased virulence resulting from the synergistic association of the two viruses may be limited to *P. unipuncta*.

There is a possibility that the phenomenon of latent virus may be involved in the present study. Bergold (1951) reported that the granulosis virus of *Cacoecia murinana* (Hb.) was not lethal to the spruce budworm but that this virus did activate a latent polyhedrosis. The present case, however, differed from Bergold's observation in that: (1) the granulosis virus was directly infectious to *P. unipuncta*, (2) no latent polyhedrosis developed in larvae fed the granulosis virus alone, and (3) larvae infected by a mixture of polyhedrosis and granulosis viruses occasionally had both polyhedra and granules within their tissues.

The present study is rather limited in scope and factors, such as physical, genetic, and physiological, which are known to affect host susceptibility have not been investigated. These factors have been discussed by Bergold (1943, 1953), Steinhaus (1949, 1954), and Vago (1951). It is apparent from this study, however, that the age of larva and the synergistic relationship of the two viruses are among the most important factors affecting the susceptibility of the armyworm to virus infections.

The synergistic association of the two viruses was probably one of the major factors responsible for the virus epizootic observed among the armyworms in the field. This has been further indicated by a second observation made during May 1955, when another outbreak of *P. unipuncta* occurred in Kohala, Hawaii. Again the outbreak was in part, if not mainly, controlled by a virus epizootic. Field-collected armyworms were found infected by both polyhedrosis and granulosis viruses. Out of 27 larvae, 9 had polyhedra, 12 had granules, and 6 had both types of inclusion bodies in them. One may conjecture that the armyworm population in the field at first acquires one of the viruses which results in an enzootic, and that later infection by a second virus creates an epizootic. Further observations are necessary to substantiate this hypothesis.

SUMMARY.—Although the armyworm, *Pseudaletia unipuncta*, appeared highly susceptible to virus infection under field conditions, it was resistant under laboratory conditions to a polyhedrosis and a granulosis virus which were isolated from field-infected larvae. The following four factors were investigated in the laboratory to determine whether they significantly affected the susceptibility of the armyworm: (1) the age of larvae, (2) multiple virus infection, (3) dosage of virus inoculum, and (4) different types of food.

Resistance of the armyworm to virus infections increased directly with the age of larvae. First- and second-instar larvae were highly susceptible to both viruses, whereas the last four instars were much more resistant. The armyworm appeared to be more susceptible to granulosis virus than to polyhedrosis virus.

In the present study, the most important factor that affected the susceptibility of the armyworm to virus infections was the synergistic association of the two viruses. A mixture of the two viruses when fed to the more resistant last four larval instars resulted in high mortality from virus infection. The infected larvae exhibited typical symptoms of polyhedrosis and their tissues after death contained mainly polyhedra; but some larvae had both polyhedra and granules, which indicated that a multiple virus infection had occurred. This is apparently the first record of a synergistic relationship between two insect viruses which results in increased virulence of the pathogens. The synergistic association was probably one of the major factors responsible for the virus epizootic observed among armyworms in the field.

Heavy virus concentrations and the repeated feeding of each virus over a period of 11 to 12 days did not markedly affect larval susceptibility. Feeding the larvae Napier grass, Kikuyu grass, or corn, and the sudden change in food from Napier grass to Kikuyu grass, from Napier grass to corn, or from corn to Napier grass also had little effect on larval susceptibility.

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Effects of Demeton and Schradan on *Peregrinus maidis* (Ashm.) and Its Egg-predator, *Cyrtorhinus mundulus* (Breddin)[1]

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Several workers have reported success in controlling aphids, coccids and red spider mites by using systemic insecticides. Ivy *et al.* (1950) soaked cotton seeds in 2, 1, and 0.5 per cent water solutions of schradan for 2 hours and found this treatment to be effective against spider mites on plants in the seedling stage. Tsi (1950) obtained mortality of aphids on young bean plants for 50 days after seeds were soaked with schradan for 24 hours at a concentration of 0.5 per cent. Dowdy & Sleesman (1952) obtained good initial kill of the potato leafhopper, *Empoasca fabae* Harris, with Systox. Reynolds *et al.* (1953) obtained excellent control of the cabbage aphid, the pea aphid and the green peach aphid with Systox applications but failed to obtain good control of the onion thrips, cyclamen mite and the white fly, *Trialeurodes abutilonea* (Haldeman).

Ripper *et al.* (1951) concluded that systemic insecticides, in general, make plants toxic to sucking pests without making them toxic to beneficial insects. Such insecticides, they argued, could be used to supplement the biological control of insect pests. Hanna *et al.* (1952) explained the reduction of Coccinellid predators of mealy bugs on cacao, after the use of a systemic insecticide, on the basis of severe reduction of the population of the mealy bugs. Ahmed *et al.* (1954) provided cotton aphids, poisoned by feeding on leaves of Systox-treated cotton plants, to several species of predators and found three species of Syrphidae highly susceptible in larval stages. Adults of Coccinellidae and larvae of two species of *Chrysopa* were generally not affected.

Peregrinus maidis (Ashm.), the corn leafhopper, is a serious pest of corn, *Zea mays* L., in Hawaii. *Cyrtorhinus mundulus* (Breddin), a predaceous Mirid bug, which was originally introduced in Hawaii for the biological control of the sugarcane leafhopper, *Perkinsiella saccharicida* Kirk. (Muir 1920) and which it successfully controlled, lives on the eggs of the corn leafhopper, which are laid usually in the midribs of corn leaves. However, the biological control of the corn leafhopper has not been very successful in Hawaii. With a view to possibly supplement the biological control of this leafhopper with chemicals, investigations were made with two systemic insecticides, Systox,[4] a formulation containing demeton, and schradan.[5] Laboratory studies were made to determine the comparative contact toxicity of the two insecticides to the two species as well as the systemic action from seed, foliage and root treatments. Demeton was also tried in the field to compare its effect on the population of the two species with that of DDT.

PROCEDURE.—For contact toxicity, the two insecticides, diluted in water, were applied to CO_2-anesthetized insects by means of a spray-tower (Ebeling 1953). The working of this equipment is based on the venturi principle. Five mls. of the insecticidal formulations were introduced from the bottom and the resultant mist was allowed to settle on the insects in petri dishes for 2 minutes after which they were transferred to glass jars and provided with their respective food. Concentrations were calculated on the basis of active ingredients per ml. of the diluted material. Mortality counts were made 24 hours after treatment and all calculations as to $L.C._{.50}$ values and their fiducial limits were made according to Finney (1947). The formula of Abbot (1925) was invariably used throughout the present study to calculate corrected mortality percentages.

Corn seeds of the variety U.S.D.A.34 were soaked in three concentrations of each insecticide for 18 hours before sowing. Just before sowing the seeds were dipped in fresh water to remove any insecticide adhering to the seed coat. After the seeds had germinated and the plants had developed a few leaves each, some of the latter were placed in glass jars. Adult leafhoppers were kept on them for 24 hours before mortality counts were made.

Three concentrations of each insecticide were used to spray one-half each of several potted plants by means of a hand atomizer. The other half of each plant was covered with a paper bag and tagged. Care was taken to prevent contamination of the soil in the pots by the insecticides. The amount of diluted material used to spray one-half of each plant was 40 mls. The toxic effects of the directly sprayed leaves as well as the unsprayed ones of the same plant were tested on leafhoppers in glass jars.

For root treatments, corn was planted in pots without any holes at the bottom. When the plants were 3 weeks old, they were infested with adult corn leafhoppers for oviposition to ensure food supply for *C. mundulus* later on. The dose of each insecticide was calculated on the basis of mgs. of active ingredients per plant and applied to the pots in 50 mls. of irrigation water. To test the toxic effects of the treated corn on *P. maidis*, leaves were provided to them in glass jars. Midribs of leaves from treated plants, containing *P. maidis* eggs were likewise provided to *C. mundulus*. Mortality counts were usually made after 24 hours, unless otherwise stated. To determine the mode of entry of the insecticides from the treated corn, adult leafhoppers were debeaked in order to prevent feeding. Debeaking was accomplished with a fine pair of tweezers. The *P. maidis* beak is somewhat brittle and squeezing the beak of a CO_2-anesthetized insect removes it quite satisfactorily. This process apparently did not affect any of the normal activities of the insects, except feeding, for at least 24 hours. In order to see whether the insecticides persisted in the soil

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in the pots, the corn plants were removed after they were ripe and new corn was planted in the same pots, After the second series of corn was about a month old, about 3 months after the original insecticidal application, leaves were tested for their possible toxic effects on *P. maidis*, as usual.

To compare the effect of demeton on the population of the two species in the field with that of DDT, a field 150 feet by 30 feet was planted with corn on January 14, 1954, in 10 rows, 3 feet apart. The field was divided into 9 experimental plots in a 3 by 3 Latin square arrangement. Each experimental plot had two rows and was separated from others by a guard row. One row was left on either of the longer sides of the field as a border. Because of heavy and practically continuous rains throughout the month of February, the development of the leafhopper population was retarded. The low leafhopper population and the rainy weather caused the insecticidal application to be delayed until March 6, 1954, approximately 7 weeks after planting, when a single application each of demeton, at 0.4 lbs. per 100 gallons, and of 50% DDT, at 2 lbs. of wettable powder per 100 gallons, was made with a 4-gallon knapsack sprayer. A pre-treatment sampling of the leafhopper and *C. mundulus* populations was made on March 5, 1954, followed by weekly samples until the corn was ripe. In order to estimate the *P. maidis* population, four plants were selected at random from each experimental plot. The plants were cut into pieces of suitable size, sealed in marked paper bags, brought to the laboratory, where 2nd to 5th instar nymphs were counted after they were killed with chloroform. The fact, that most of the nymphs are found under the leaf sheaths, prevented any significant escape. In order to estimate the *C. mundulus* population, nymphs of all stages were counted in the field from four plants selected at random from each experimental plot.

Table 1.—Contact toxicity of demeton and schradan to *P. maidis* and *C. mundulus*.

Insecticide	Conc. Mg./Ml.	No. of Insects Treated	Corrected Per Cent Mortality	L.C.$_{50}$ Mg./Ml.	Fiducial Limits p=0.05
		Peregrinus maidis			
Demeton	0.10	40	100		
	.08	43	100		0.046
	.06	88	89	0.044	
	.05	89	71		
	.04	85	37		.041
	Untreated	60	5		
Schradan	6.20	41	100		
	1.24	71	100		0.592
	0.99	73	98	0.580	
	.74	63	78		
	.50	83	31		.569
	Untreated	60	5		
		Cyrtorhinus mundulus			
Demeton	0.08	42	82		
	.06	52	74		0.044
	.04	45	47	0.037	
	.02	48	20		
	.01	47	14		.031
	Untreated	33	3		
Schradan	7.44	42	100		
	4.96	31	93		1.990
	2.48	92	67	1.773	
	1.24	83	30		
	0.99	50	20		1.578
	Untreated	46	4		

Table 2.—Corrected per cent mortality in *P. maidis* obtained by seed treatment.

Insecticide	Concentration in Mgs. per Ml.	Days After Treatment				
		10	12	18	28	42
Demeton	10.0	100	100	85	75	25
	1.0	96	38	40	35	4
	0.1	40	27	0	—	—
Schradan	63.0	40	60	20	0	—
	31.5	48	33	19	3	—
	12.6	20	0	—	—	—
Untreated	—	0	0	0	8	0

DISCUSSION OF RESULTS.—Table 1 summarizes the contact toxicity of demeton and schradan to the two species. There were no significant differences between the susceptibilities of the two species to demeton, as the fiducial limits of the L.C.$_{50}$ values of this insecticide, against the two species, overlapped. Schradan, on the other hand, was found to be approximately three times as toxic to *P. maidis* as to *C. mundulus*. One concentration of schradan, 0.99 mg. per ml., caused 98 per cent mortality in *P. maidis* but could not cause more than 20 per cent mortality in *C. mundulus*. Comparisons of the two insecticides showed that demeton was approximately 13 times as toxic as schradan to *P. maidis* and 48 times as toxic to *C. mundulus*. Demeton also acted much faster than schradan. The higher concentrations of demeton caused 100 per cent mortality in *P. maidis* within 20 minutes after application. Comparisons with other insecticides tested against the same species (Verma 1954) showed that demeton was about one-half as toxic as parathion and approximately 3 to 4 times as toxic as p-p′-isomer of DDT.

The results of seed treatments are summarized in table 2. All the treated seeds gave rise to plants that were toxic to *P. maidis* in varying degrees. However, the only concentration that showed signs of considerable effectiveness against the leafhopper was 10 mgs. of demeton per ml. The plants in this case remained toxic up to 6 weeks after planting. Schradan did not appear to be an effective seed treating material for corn. Germination was delayed by about 2 days in the case of seeds soaked in 10 mgs. of demeton per ml. After germination, all the plants grew approximately at the same rate. However, phytotoxic symptoms in the form of banded chlorosis (Fig. 1) appeared at the center of certain leaves of plants, developing from seeds soaked in higher concentrations of both insecticides.

Table 3 summarizes the results of foliage treatments. There was practically uniform distribution of demeton in the plants within 24 hours, at concentrations of 1 and 0.1 mg. per ml. Both sprayed and unsprayed leaves

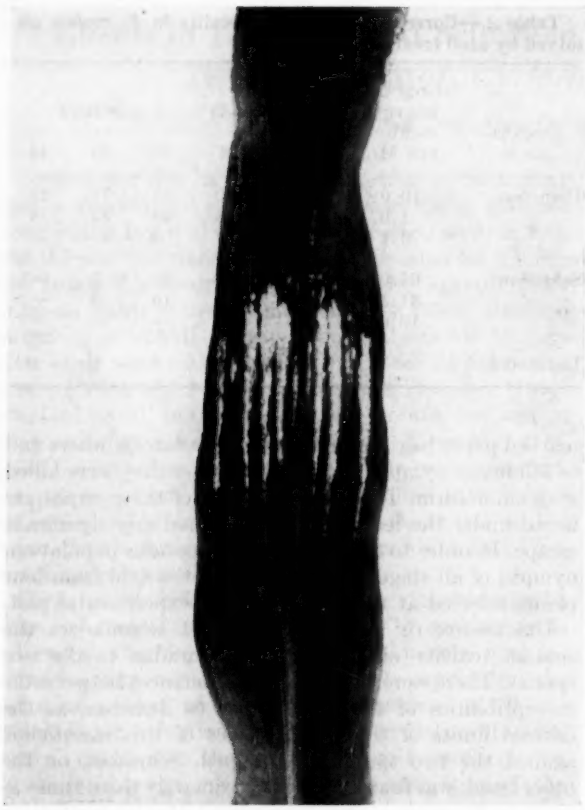

FIG. 1.—Corn leaf showing phytotoxic symptoms in the form of banded chlorosis caused by seed treatment with demeton.

caused 100 per cent mortality 24 hours after treatment, when 1 mg. of demeton per ml. was used. There was no translocation of demeton, in toxic amounts, to unsprayed leaves from leaves sprayed with 0.01 mg. per ml. Translocation appeared to be slower in schradan-treated plants. Leaves sprayed with schradan at a concentration of 13 mgs. per ml. caused 100 per cent mortality in *P. maidis*, 24 hours after treatment. On the other hand, unsprayed leaves of the same plants caused only 50 per cent mortality. However, the sprayed and unsprayed leaves were equally toxic 11 days after treatment. In the lower concentrations, schradan could not be translocated in toxic amounts from sprayed leaves to unsprayed ones.

Table 3.—Corrected per cent mortality in *P. maidis* obtained by foliage treatments.

Insecticide	Concentration in Mg. per Ml.	Types of Leaves	Days After Treatment		
			1	11	21
Demeton	1.0	Sprayed	100	67	12
	—	Unsprayed	100	44	13
	0.1	Sprayed	71	10	0
	—	Unsprayed	78	6	3
	0.01	Sprayed	36	15	0
		Unsprayed	0	18	0
Schradan	13.0	Sprayed	100	83	10
	—	Unsprayed	50	85	17
	1.3	Sprayed	94	18	0
	—	Unsprayed	0	5	0
	0.13	Sprayed	33	10	0
	—	Unsprayed	10	5	0
Untreated	—	—	0	0	8

Table 4.—Corrected per cent mortality in *P. maidis* caused by root treatments.

Insecticide	Dose in Mgs. per Plant	Days After Treatment					
		2	7	14	21	26	40
Demeton	500.0	100	100	100	100	Plant Died	—
	50.0	100	100	100	96	86	47
	25.0	100	100	89	75	73	13
	12.5	100	100	67	47	5	—
	5.0	100	91	33	25	0	—
	0.5	14	5	0	0	0	—
Schradan	1257	100	100	100	93	84	Plant Died
	943	100	100	100	93	86	95
	628	100	100	100	94	79	89
	314	95	100	92	73	83	80
	63	47	88	90	58	27	47
Untreated	—	0	0	0	0	8	0

Table 4 presents the mortality percentages in *P. maidis* caused by demeton and schradan when used as root treating materials. Both insecticides appeared to be readily absorbed by the root system of corn and translocated to all the parts within 48 hours. As low a dose of demeton as 5 mgs. per plant caused 100 per cent mortality 2 days after and 91 per cent mortality 7 days after treatment. Schradan appeared to be somewhat less efficient but this insecticide persisted longer in the plants. Forty days after treatments, some of the plants died while others were practically mature and dry. Table 5 summarizes the toxic effects of demeton and schradan treatments on *C. mundulus*. The two insecticides differ appreciably in their toxicity to this predator. Whereas demeton at 25 mgs. per plant caused 100 per cent mortality 1 day after treatment, schradan at 1257 mgs. per plant caused only 10 per cent mortality. However, in the case of demeton treatments, the toxic effect on *C. mundulus* appeared to fade fast. Seven days after treatment the mortality rate was very low. On the other hand, schradan-treated plants were practically non-toxic to *C. mundulus* throughout.

It was suspected that the eggs of *P. maidis*, laid inside the tissues of corn leaves, might have been poisoned by demeton and not by schradan. However, there was

Table 5.—Corrected per cent mortality in *C. mundulus* caused by root treatments.

Insecticide	Dose in Mgs. per Plant	Days After Treatment	
		1	7
Demeton	25.0	100	10
	12.5	80	25
	5.0	20	0
	2.5	20	10
	1.3	0	0
Schradan	1257	10	0
	943	5	10
	628	0	—
	314	0	—
	63	0	—
Untreated	—	0	0

Table 6.—Toxicity of demeton- and schradan-treated corn to normal and debeaked *P. maidis*.

TREATMENT[a]	CONDITION OF *P. maidis*	PER CENT MORTALITY IN 12 HOURS
D_1	Normal	100
D_1	Debeaked	100
D_2	Normal	71
D_2	Debeaked	4
D_2 A.T.[b]	Normal	96
D_2 A.T.	Debeaked	47
S	Normal	69
S. A.T.	Debeaked	8
Untreated	Normal	0
Untreated[c]	Debeaked	0

[a] D_1=Demeton treatment at 500 mgs. per plant 2 weeks previously.
D_2=Demeton treatment at 50 mgs. per plant 1 week previously.
S=Schradan treatment at 1257 mgs. per plant 2 weeks previously.
A.T.=Air tight jar, ordinarily cheesecloth being used.
[b] Kept for only 6 hours.
[c] Kept for 24 hours.

Table 7.—Persistence of demeton and schradan in the soil as judged by mortality in *P. maidis* and the number of ovipositional scars produced in the second series of corn.

INSECTICIDE	PREVIOUS DOSE IN MGS. PER PLANT	PER CENT MORTALITY in *P. maidis*	NUMBER OF OVIPOSITIONAL SCARS PRODUCED IN 3 DAYS
Demeton	50.0	0	140
	25.0	0	66
	12.5	0	87
	5.0	0	—
	0.5	0	—
Schradan	1257	26	7
	943	38	0
	628	61	2
	314	16	—
	63	4	24
Untreated	—	0	134

normal hatching of *P. maidis* from plants of both types of treatment, showing that the eggs were not poisoned. If adults of *C. mundulus* were kept in glass jars with corn midribs containing *P. maidis* eggs from untreated plants together with leaves, without any eggs, from demeton-treated plants, mortality was equally high. Leaves from schradan-treated plants provided likewise failed to cause any mortality. These observations showed that demeton from treated plants affected *C. mundulus* not by poisoning the food supply. Laboratory studies have shown that whereas demeton is a good fumigant, schradan at best is a very poor one. Also, studies on the mode of entry of these two insecticides from treated plants, by using normal and debeaked *P. maidis*, have shown that demeton does affect by fumigant action whereas schradan does not.

Table 6 summarizes the results of the study on the mode of entry. When leaves from plants, treated with 500 mgs. of demeton 2 weeks previously, were tested there was 100 per cent mortality in both normal and debeaked *P. maidis*. Leaves from untreated plants failed to cause any mortality in debeaked insects. When leaves from plants, treated with 50 mgs. of demeton 1 week previous to the test, were provided to normal insects, there was 71 per cent mortality. However, such leaves failed to cause any noticeable mortality in debeaked insects. Further, when the jars were kept air tight, the same leaves caused 96 per cent mortality in normal *P. maidis* in only 6 hours and 47 per cent mortality in debeaked insects in 12 hours. These observations left no doubt that leaves from plants grown in soil treated with demeton had fumigant effect also. Leafhoppers were affected both by their feeding activities on such leaves as well as by the toxic vapors emitted. This fumigant effect became less marked after some days depending, among other factors, upon the amount of the insecticide applied to the plants. When debeaked insects were kept in air-tight jars containing leaves from schradan-treated plants, there was practically no mortality. However, 69 per cent of the normal insects died on the same leaves even when the jars were simply covered with cheese cloth. It is clear, therefore, that schradan from the leaves of treated plants did not affect the insects by fumigant action. It was noticed that both young and old leaves from both types of treatment were practically equally effective.

No trace of the persistence of demeton was found in the soil, as judged by the per cent mortality in *P. maidis* caused by feeding on the second series of corn, 3 months after the original and only insecticidal application was made. Schradan, however, showed some persistence (Table 7). Some of the potted plants of the second series from each type of treatment were kept in a corn field having a high leafhopper population. After 3 days the ovipositional scars were counted. It is shown in Table 7

Table 8.—Effect of demeton and DDT on the populations of *P. maidis* and *C. mundulus*.

INSECTICIDE PER 100 GALLONS	DATE OF SAMPLING											
	March 5[a]		March 12		March 19		March 27		April 19		April 16	
	P.m.[b]	C.m.[c]	P.m.	C.m.	P.m.	C.m.	P.m.	C.m.	P.m.	C.m.	P.m.	C.m.
Untreated	65	0	82	1	70	9	83	17	135	24	196	85
Demeton, 0.4 lb.	99	0	2	0	14	8	38	6	97	0	183	5
DDT, 50%, 2 lbs. W.P.	71	0	19	0	11	0	4	0	24	0	107	1
L.S D. at 5% level	—	—	49	—	42	—	38	—	28	—	n.s.	—

[a] Pretreatment sampling.
[b] *P. maidis* population per plant.
[c] *C. mundulus* population per 12 plants.

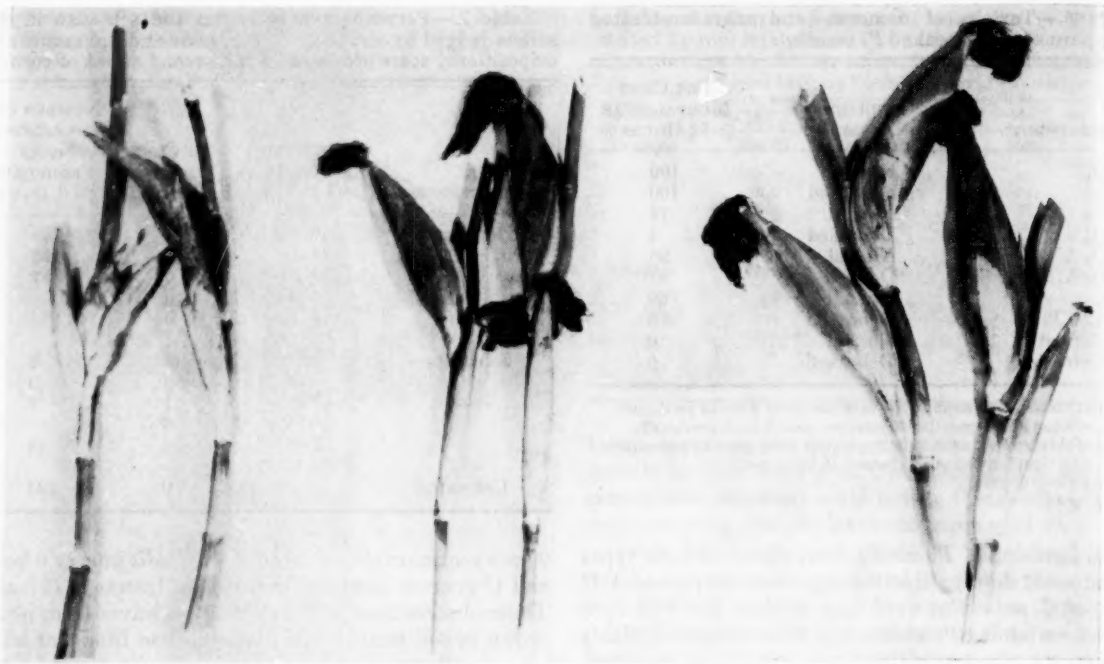

FIG. 2.—Ears of corn taken from demeton-sprayed plots (left) together with those from unsprayed (center) and DDT-sprayed plots (right). Note the absence of silk in demeton-sprayed ears.

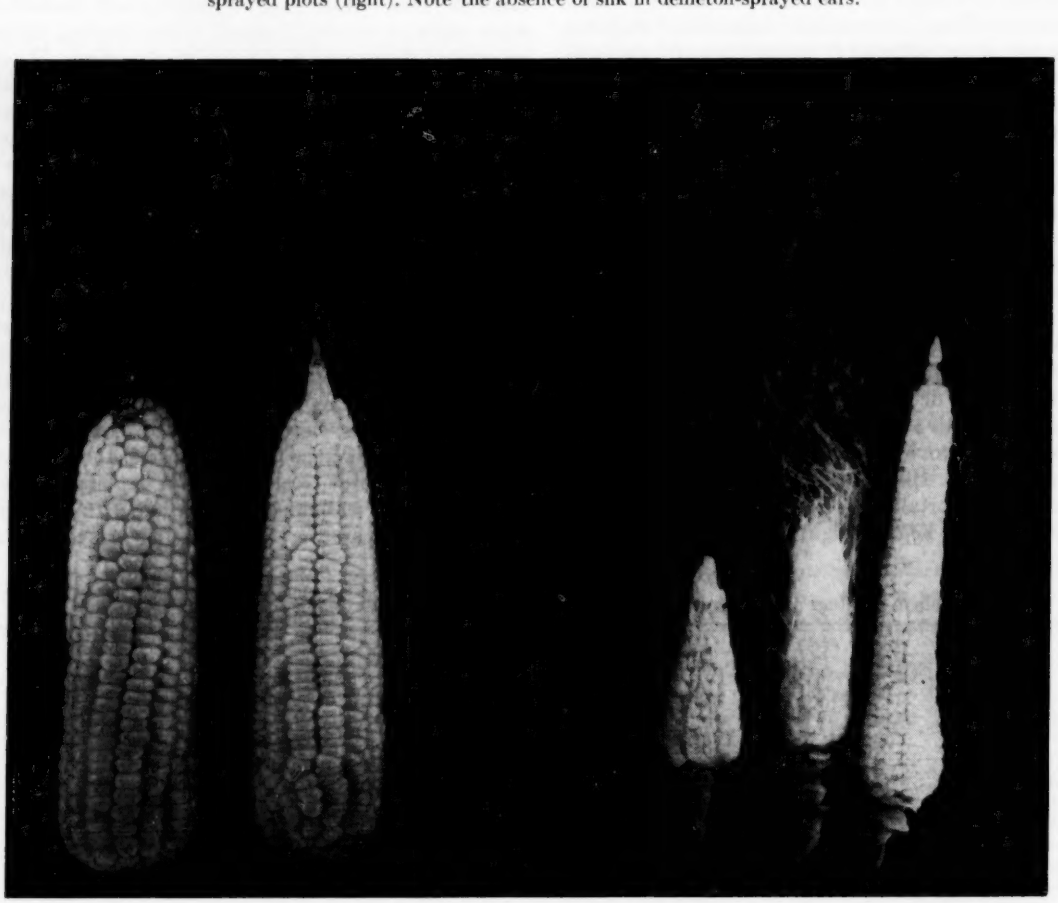

FIG. 3.—Healthy cobs (left) and undeveloped cobs from demeton-sprayed plots (right), opened up to show kernels. Note that no kernels are formed in demeton-sprayed ears.

that whereas plants grown in soil previously treated with demeton, as in the control, had large numbers of ovipositional scars, there were very few in plants growing in soil previously treated with schradan. It appears possible that the females could detect the toxic properties of these plants before ovipositing in them.

Phytotoxic symptoms developed in the treated plants Plants grown in soil treated with 500 mgs. of demeton per plant showed marked stunting, generalized yellowish orange coloration, severe burning of the tips and margins of the leaves and dried up within 3 weeks after treatment. Plants grown in soil treated with 50 mgs. of demeton or 1257 mgs. of schradan per plant developed banded chlorosis near the center of the leaves, similar to that produced by seed treatments.

The effect of a field application of demeton on the populations of the two species, as compared with that of DDT, is presented in Table 8. One week after spraying demeton caused a 98 per cent reduction in the population of the leafhopper. Soon thereafter, the population started to increase. DDT, on the other hand, caused a 73 per cent reduction in the leafhopper population, 1 week after treatment. The population in this case, however, continued to decrease until 3 weeks after treatment. This can, perhaps, be explained on the basis of the systemic and residual behaviour of demeton and DDT respectively, as also the fact that most of the nymphs are found under tight leaf sheaths and at least some of them can always escape conventional types of insecticides. Table 8 also shows the comparative build-up of *C. mundulus* populations, which remained practically nil in DDT-sprayed plots. In demeton-treated plots also, it remained extremely low. Demeton, thus, did not show any signs of supplementing the biological control of *P. maidis*.

Serious phytotoxic symptoms appeared on demeton-sprayed plants. These plants were slightly stunted and tasselling was delayed. A great majority of the plants failed to produce healthy ears. Figure 2 illustrates the ears taken from plots sprayed with demeton or DDT and from unsprayed plots. The breaking out of the stigmal hairs or silk appeared to be seriously affected. In all instances in which the ears were not developed, the stigmal hairs failed to come out of the shuck. Figure 3 shows the healthy and undeveloped cobs. It is clear that the silk, even though quite well-developed, failed to extend beyond the shuck. This failure might have been responsible for the undeveloped kernels. On an average, only 12 to 15 per cent of the demeton-sprayed plants could produce healthy ears. In all of these cases, the stigmal hairs extended normally beyond the shuck.

SUMMARY.—Contact toxicity of demeton and schradan to *Peregrinus maidis* and its egg-predator, *Cyrtorhinus mundulus* was determined. Demeton was equally toxic to the two species but schradan was about three times as toxic to the pest as to its predator. Demeton was much more toxic than schradan to both the insects.

Corn seeds soaked in several concentrations of each insecticide before planting gave rise to plants toxic to the leafhoppers. Demeton proved more effective than schradan. From foliage sprays both insecticides were translocated to parts that were not sprayed. Demeton was translocated faster than schradan.

Both insecticides were readily absorbed by the root system of corn and translocated to all the aerial parts. Demeton proved more effective than schradan against the leafhopper but affected *C. mundulus* by fumigation through the leaves. Schradan persisted longer in the soil than demeton.

A preliminary field trial of demeton was made to compare its effect on the population of the two species with that of DDT. DDT controlled the leafhopper population longer than demeton. Demeton affected the predator population almost as seriously as DDT. Spraying with a concentration of 0.4 lb. of demeton per 100 gallons caused serious phytotoxicity.

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Treated Cloth Bags to Control the Rice Weevil in Corn

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Treatment of cloth storage bags with an insecticide may be an important adjunct in controlling insect pests in stored grain products and seed. Cotton *et al.* (1949) have shown the protection afforded to flour from insect attack when it was stored in cotton bags impregnated with pyrethrins alone or mixed with piperonyl butoxide. Atkins & Greer (1953) tried 0.1% BHC and 1.0, 1.25, and 1.5% DDT impregnated or sprayed on jute bags used for flour storage. The 1.5% DDT inpregnated bags gave protection against *Ephestia kuhniella*, *Ptinus tectus*, and *Tribolium confusum*. In all cases the amount of insecticide which penetrated into the flour was not higher than the tolerance permitted in human food. Nakajima (1952) reported that rice stored in bags treated with 1% purified BHC was not infested with rice weevils. There is some possibility that higher dosages of certain insecticides may result in an undesirable insecticidal residue odor, or off-flavor in a product stored in a treated bag. This problem, however, is not so important when seed is stored in treated bags. Jewett & Price (1951) impregnated or sprayed bags with 10% DDT dissolved in carbon tetrachloride. Seed corn stored in treated bags was protected against attack by numerous storage pests for 18 months. Lower concentrations of DDT were not satisfactory.

Bag treatment for protection of stored seed against insect attack has some advantages over other methods of control. A small amount of insecticide is used. Not all of the seed comes in contact with the treated bag, thus the possibility of undesirable odor and the deleterious effect on seed germination is lessened. Furthermore, a long period of protection may be effected without the necessity of retreating the seed.

The present paper is a summary of tests conducted to evaluate the degree of insect control obtained by storing corn in treated bags.

METHODS AND MATERIALS.—Heavy cotton twill bags, 18 inches long and 7½ inches wide were sprayed with a 1% dilution of the following insecticides:[2] aldrin, BHC, dieldrin, endrin, isodrin, lindane, malathion, parathion and Strobane. The diluted formulations were prepared from emulsifiable concentrates, except for parathion, which was prepared from a wettable powder concentrate. Sprays were applied with a hand atomizer[3] to the inside of the bags at the rate of 10 ml. per bag. Bags were allowed to dry before they were filled with 10 pounds of untreated, shelled corn.

In addition to the sprays, two dust formulations were used. One was a wettable powder containing 10% piperonyl butoxide and 0.75% pyrethrins. The other dust was a 100% concentration of Ryania. Dusts were mixed with corn at the rate of 10 grams of dust per 10 pounds of shelled corn and stored in untreated bags. In the untreated checks neither the corn nor the bags were treated.

Each treatment was replicated four times. Each replicate consisted of one bag containing 10 pounds of shelled corn. The corn was stored for a period of 6 months beginning May 1, 1954, and ending November 11, 1954, in an earthen-floored basement room where the temperature averaged 79° F. and the relative humidity 81 per cent.

A yellow corn hybrid, N.C. 27, harvested in the fall of 1953, was used in all treatments. The corn was kept at least 4 months after harvest before it was used in these tests. By this time, rice weevils were evident, presumably developing from an original field infestation. The moisture content of the corn averaged 13.75 per cent during the test.

All treatments were examined at 1, 2, 3 and 6 months after treatment to check on insect development and the condition of the corn. At each examination, a sample of one pint of corn was pulled at random from each bag and the number of insects in this sample was recorded. In addition, 100 kernels were selected at random from each pint sample and stained according to the acid-fushsin method described by Frankenfeld (1948).

RESULTS.—The results of the examination of the pint samples are presented in table 1. Rice weevils were the only insects found in these samples. All treatments had a few rice weevils at 1 month after treatment. At 3 months after treatment, live weevils were found in the parathion, pyrethrins,-piperonyl butoxide, Strobane treatments as well as in the untreated check. At 6 months after treat-

Table 1.—Average number of dead and live rice weevils per pint of shelled corn in treated bags.

	Before Treatment		After Treatment[a]							
	May 3		June 1		July 5		Aug. 9		Nov. 11	
	D	L	D	L	D	L	D	L	D	L
Aldrin	1	3	2	0	4	0	3	0	4	0
BHC	0	3	3	0	3	0	1	0	2	0
Chlordane	1	3	2	1	2	0	3	0	1	0
Dieldrin	1	1	2	0	5	0	3	0	1	0
Endrin	1	3	2	1	7	0	2	0	3	0
Heptachlor	2	4	5	1	8	0	1	0	7	0
Isodrin	0	2	2	0	6	0	2	0	0	0
Lindane	1	2	2	0	6	0	3	0	2	0
Malathion	3	3	4	0	6	0	3	0	7	0
Parathion	1	2	1	1	6	1	4	1	6	0
Pyrethrins-piperonyl butoxide[b]	1	2	3	0	7	0	7	1	12	1
Ryania[b]	1	3	2	0	8	1	6	0	4	0
Strobane	1	4	3	1	3	2	4	4	1	375
Check	1	2	1	2	2	10	2	24	4	384

[a] D=dead; L=live.

[b] Treated corn stored in untreated bags; all others, except check, untreated corn stored in treated bags.

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[2] Aldrin, dieldrin, endrin and isodrin supplied by the Shell Chem. Corp., Denver, Colo.; chlordane and heptachlor, by the Velsicol Corp., Chicago, Ill.; malathion and parathion by American Cyanamid Co., New York; Strobane by the Goodrich Co., Cleveland, Ohio; pyrethrins-piperonyl butoxide by the Fairfield Chemical Division, Food and Machinery Corp., Baltimore, Md.; and Ryania by S. B. Penick and Co., New York.

[3] A DeVilbiss No. 15 atomizer.

Table 2.—Rice weevil damage per 100 kernels corn.

Material	Before Treatment	After Treatment											
	May 3	June 4			July 5			Aug. 9			Nov. 11		
	E F X[a]	E F X			E F X			E F X			E F X		
Aldrin	0 2 2	0 3 3			0 6 4			0 4 2			0 5 1		
BHC	0 1 0	0 1 0			0 2 1			0 3 1			0 3 2		
Chlordane	0 2 1	0 1 1			0 4 2			0 3 2			0 2 0		
Dieldrin	0 2 0	0 3 1			0 4 1			0 3 1			0 2 1		
Endrin	0 2 0	0 3 1			0 4 1			0 2 0			0 3 1		
Heptachlor	0 2 1	0 2 3			0 3 1			0 3 2			0 5 1		
Isodrin	0 2 0	0 1 0			0 5 3			0 3 1			0 1 0		
Lindane	0 2 1	0 2 1			0 6 2			0 2 0			0 2 0		
Malathion	0 2 1	0 2 3			0 1 2			0 2 1			0 3 1		
Parathion	0 1 0	0 1 0			1 3 4			0 2 2			0 3 2		
Pyrethrins-piperonyl butoxide	0 2 1	0 1 0			0 3 4			0 2 2			0 4 1		
Ryania	0 2 1	0 2 3			1 1 1			0 2 2			0 1 1		
Strobane	0 3 1	0 3 2			0 2 1			0 4 2			8 61 46		
Check	0 2 2	1 3 2			4 12 10			2 9 6			10 56 51		

[a] E—Egg plugs.
F—Feeding punctures.
X—Emergence holes.

ment only three treatments had any live weevils, namely; pyrethrins-piperonyl butoxide, Strobane, and untreated check—the number of weevils in the last two treatments had increased markedly.

The stain counts listed in table 2 paralleled the weevil counts of table 1. Where the weevil population was high, there was a correspondingly high count of egg laying and feeding punctures as well as weevil exit holes.

SUMMARY.—One per cent dilutions of 11 different insecticides were sprayed on the inside of heavy cotton twill bags filled (after drying) with untreated yellow shelled corn (hybrid N.C. 27). Two dusts—one a combination of pyrethrins and piperonyl butoxide, and the other, Ryania, were applied directly to corn and mixed with it prior to storage in untreated bags. Six months after treatment, there was no marked increase in weevil development in any of the bags except in the Strobane-treated bag and in the untreated check.

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Further Laboratory Tests for Wireworm Control[1]

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The development of new insecticides and different formulations has stimulated continued research in the control of wireworms. Previous laboratory tests for wireworm control in North Carolina have been summarized in a report on insecticidally treated seeds (Kulash 1953) and in a report on different methods of control (Kulash & Monroe 1954). The present paper is a summary of additional tests with treated seed and treated soil for control of wireworms attacking corn.

MATERIALS[2] AND METHODS.—Nine insecticides were used in two ways (a) as sprays formulated from wettable powder or emulsifiable concentrates and (b) as impregnations on citrus pulp. These insecticides were used as seed and soil treatments. One insecticide, a 25 per cent wettable powder concentrate of 2,4-dinitrofluorobenzene, was used as a seed coating only. A second insecticide, 0.08 per cent pyrethrum-1.1 per cent piperonyl butoxide dust, was used as a combination treatment wherein both the seed and the soil were treated in the same test. The remaining seven materials were used separately in seed and soil treatments. These materials are described as follows:

Dieldrin-thiram wettable powder—12.5 per cent dieldrin, 56.25 per cent tetramethylthiuram (thiram), 31.25 per cent inert ingredients.

Lindane-thiram wettable powder—14 per cent BHC (from lindane), 56 per cent thiram, 30 per cent inert ingredients.

Five per cent aldrin, chlordane, dieldrin, and heptachlor impregnated on citrus pulp hammer mill ground to particle size large enough to pass through 50–100 mesh screens.

Endrin emulsifiable concentrate, 18.5 per cent.

Dependent upon the formulation used, seed coatings were applied in two different ways. The wettable powders and emulsifiable concentrate were first diluted with 5 ml. of water and then mixed thoroughly with 100 grams of clean corn seed. The mixing was done by stirring the seed in a small breaker with a spatula. The citrus pulp formulations were applied to each seed separately by first dipping each seed into a 5% Methocel[2] sticker and

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[2] The materials used in this study were supplied by the following companies:
2,4-dinitrofluorobenzene (Compound NP 1083) by the Pennsylvania Salt Manufacturing Co., Philadelphia, Pa.
0.08 pyrethrins-1.1 per cent piperonyl butoxide (Compound T-483) by the Fairfield Chemical Division of the Food Machinery and Chemical Corporation, Baltimore, Md.
Dieldrin-thiram and lindane-thiram wettable powder concentrates, by the E. I. duPont de Nemours Co., Inc., Wilmington, Del.
Aldrin and dieldrin citrus pulp formulations and emulsifiable concentrate endrin, The Shell Chemical Corporation, Denver, Colo.
Chlordane and heptachlor citrus pulp formulations, by the Velsicol Corp., Chicago, Ill., through the courtesy of the Florida Agriculture Supply Co., Jacksonville.
Methocel, a methylcellulose product of 15 centipoise viscosity, by the Dow Chemical Co., Midland, Mich.

Table 1.—Effectiveness of materials in seed and soil laboratory tests for control of wireworms eight days after planting—1953.[a]

Material Used[b]	Soil Treatment		Seed Treatment	
	Kill of Wireworms[c]	Protection to Seed[d]	Kill of Wireworms	Protection to Seed
Heptachlor citrus pulp	Fair	Moderate	Good	Good
Aldrin citrus pulp	None	Fair	Good	Good
Lindane-thiram W.P.	Fair	Fair	None	Moderate
Chlordane citrus pulp	Poor	Good	Fair	Moderate
Dieldrin-thiram W.P.	Good	Good	Poor	Moderate
Dieldrin citrus pulp	Poor	Moderate	None	Good
Endrin E.C.	(1) Fair[e] (2) None	Fair Poor	None	Poor
2,4-dinitrofluorobenzene W.P.			None	Poor
Pyrethrins-piperonyl butoxide dust			None	Poor

[a] Based on 5 replicates of 5 wireworms and 5 seeds per replicate.

[b] Pounds of actual insecticide used per acre in soil treatments: 1 lb.—pyrethrins-piperonyl butoxide, endrin; 2 lbs.—lindane-thiram; 4 lbs.—dieldrin-thiram; 5 lbs.—citrus pulp formulations of aldrin, chlordane, dieldrin, and heptachlor. Grams of material per 100 grams of seed in seed treatments used as follows: 1 gram—dieldrin-thiram, endrin, lindane-thiram, 2,4-dinitrofluorobenzene and pyrethrins-piperonyl butoxide; 1.5 grams—citrus pulp formulations of aldrin, chlordane, dieldrin, and heptachlor.

[c] Kill of wireworms; poor = 4 to 15 per cent; fair = 16 to 25 per cent; good = 26 to 36 per cent.

[d] Protection to seed based on per cent of seed penetrated by wireworms; poor = 76 to 100 per cent; moderate = 25 to 75 per cent; fair = 10 to 24 per cent; and good = 0 to 9 per cent.

[e] (1) Wireworms placed on surface of soil (usual manner). (2) Wireworms placed at bottom of test can before soil was added.

then rolling in the citrus pulp formulation. By this method, each seed was covered with approximately 0.15 gram of citrus pulp formulation. Seeds were allowed to dry for a day before they were planted in gallon-size tin cans 6 inches in diameter and 7 inches deep. Five seeds were planted in each can at a depth of 2 inches. Five cans, i.e., replicates, were used for each treatment. Each can was filled with sterilized peat muck soil obtained from an area where wireworms had severely damaged corn. Full grown wireworms (*Melanotus communis* Gyll.) were obtained from this same area a few days before they were used in the test.

Soil treatments with the citrus pulp formulations were made by dusting 1 gram of the material over the soil surface at the level at which the seeds were planted. Soil treatment with wettable powder and emulsifiable concentrate formulations were made by spraying the soil at the seed planting level. Three milliliters of finished spray were used for each can. One-half of this amount (1.5 ml.) was sprayed on the seed and soil at the planting level. A 1-inch layer of soil was added to the can and treated with 1.5 ml. of finished spray and then another inch of untreated soil was added.

After the treatments were completed five wireworms were added to the soil surface of each can in all cases but one. In this case, the wireworms were placed at the bottom of empty cans before the soil was added. Cans were kept in a shaded basement room where maximum daily temperatures averaged 80° F.

Evaluating Tests.—Daily post-treatment examinations of all cans were made to observe any activity of wireworms on the soil surface. Eight days after planting, the seedlings were 7 to 8 inches tall. At this time, the cans were emptied and final observations made on stem and root growth, wireworm activity or mortality, and injury to seeds, roots or stems by wireworm feeding.

The relative effectiveness of the different materials in the two different methods of treatment was based on two criteria—(1) injury to plants by wireworms and (2) kill of wireworms. The statistical criterion used to rank the different materials was the χ^2 statistic. (See Kulash & Monroe 1954.)

Effectiveness of the materials in killing wireworms was scored as follows: poor, killed 4 to 15 per cent; fair, killed 16 to 25 per cent; and good, killed 26 to 36 per cent. Effectiveness in protecting seed against wireworm attack was scored as follows: poor, 76 to 100 per cent of seeds penetrated; fair, 25 to 75 per cent; moderate, 10 to 24 per cent; and good, 0 to 9 per cent. The kill and penetration scores are listed in table 1.

The effect of seed-coatings on seed germination was evaluated by two different germination tests. In one test, treated seeds were planted in sterilized peat muck soil. In the second test the roll paper towel method of testing was used. In this method,[3] treated seeds were placed in moistened kraft paper towels and germinated in a daylight water curtain germinator. See table 2 for results of germination tests.

Results.—In regard to kill of wireworms, only four

[3] Paper-towel germination tests were conducted by Dr. W. H. Darst, Seed Testing Division, N. C. Department of Agriculture, Raleigh.

Table 2.—Results of soil and paper-towel germination tests of corn—1953.

Material	Soil Germination				Paper-Towel Germination	
	Per Cent Germination	Aver. Stem Length (Inches)	Aver. Tap Root Length (Inches)	Condition[a] of Roots	Per Cent Germination	Condition[a] of Roots
2,4-dinitrofluorobenzene wettable powder	36	7	2	Poor	0	None
Lindane-thiram	84	6	12	Good	94	Good
Dieldrin-thiram	88	5	11	Good	97	Good
Endrin	88	5	12	Good	94	Good
Aldrin, citrus pulp	92	5	12	Good	50	Poor
Chlordane, citrus pulp	96	7	10	Good	25	Poor
Dieldrin, citrus pulp	96	4	11	Good	40	Poor
Heptachlor, citrus pulp	96	7	11	Good	40	Poor
Citrus pulp only	—	—	—	—	30	Poor
Methocel only	100	6	11	Good	92	Good
No treatment	100	7	12	Good	97	Good

[a] Based on number of rootlets and their length.

soil treatments were scored as effective. These were dieldrin-thiram, heptachlor citrus pulp, lindane-thiram, and endrin. There was no significant difference in kill among these four materials.

Seven of the soil treatment compounds were effective in protecting the seed against wireworm attack. The dieldrin-thiram treatment had no damaged seeds, but it did not differ appreciably from the citrus pulps of dieldrin, heptachlor, and chlordane—the latter three having only a few damaged seeds. These four compounds did differ from the endrin, lindane-thiram, and aldrin citrus pulp soil treatments, all of which afforded a fair amount of protection to the seed against wireworm attack.

Only three of the seed treatment compounds produced a fair or good kill of wireworms. These were the citrus pulps of aldrin, heptachlor, and chlordane. Most of the seed treatment compounds afforded moderate or good protection to the seed against wireworm attack except endrin, pyrethrins-piperonyl butoxide, and 2,4-dinitrofluorobenzene, which scored a poor rating.

In most instances there was a negative correlation between wireworm mortality and penetration of seed by wireworms—i.e., the greater the kill, the fewer seeds damaged. In some cases, however, there was no kill and few seeds were damaged. For example, in the chlordane soil treatment, 4 per cent of the wireworms were killed but only 8 per cent of the seeds were penetrated. In the dieldrin citrus pulp seed treatment, there was no kill and no seeds were damaged. In the lindane-thiram seed treatment, no wireworms were killed but 12 per cent of the seeds were penetrated. Our studies indicate no reason for these results but it may be that in these cases wireworms were repelled by these materials.

Only one of the compounds, 2,4-dinitrofluorobenzene, had a marked adverse effect on germination. In the soil germination test, 36 per cent of the seeds germinated but plants were yellow with a poor root system (see table 2); in the paper-towel test, none of the seeds germinated.

Seeds treated with citrus pulp formulations had a high percentage of germination in the soil test but a low rate in the paper-towel test. This poor germination can be ascribed to the heavy production of several fungi (species of *Asperigillus*, *Penicillium*, and *Fusarium*) that flourished in the citrus pulp. The insecticide seemingly had no effect on the fungi, and the fungi did materially reduce the germination rate in the paper-towel method of testing. All germination tests were run within 2 months after seed had been coated.

An unusually heavy development of secondary and tertiary rootlets was observed in one series of dieldrin citrus pulp-treated seeds. The rootlets had an unusually large number of hairs which created a bushy appearance. It was not possible to reproduce this condition of the root system in other tests with dieldrin citrus pulp-treated seed.

SUMMARY.—Nine materials were applied in three different formulations in laboratory seed and soil treatments for the control of wireworms (*Melanotus communis* Gyll.) attacking corn. In the soil treatments, the best kill was obtained with dieldrin-thiram wettable powder at 4 lbs./A. of dieldrin, followed in order of effectiveness by endrin, 1 lb./A.; heptachlor, 5 lbs./A.; and lindane-thiram wettable powder at 2 lbs./A. of lindane. All soil treatments gave fair or good protection to seed against wireworm attack but dieldrin-thiram at 4 lbs./A. of dieldrin and 5 lbs./A. of citrus pulp formulations of chlordane, dieldrin, and heptachlor were better than endrin at 1 lb./A., lindane-thiram at 2 lbs./A. of lindane, and 5 lbs./A. of aldrin citrus pulp. As seed treatments, the citrus pulp formulations of aldrin, heptachlor, and chlordane at 1.5 grams of 5 per cent material per 100 grams of seed gave a fair or good kill of wireworms. All seed treatments gave moderate or good protection against wireworms attacking the seed except endrin, pyrethrins-piperonyl butoxide, and 2,4-dinitrofluorobenzene.

In germination tests in sterilized soil, citrus pulp formulations did not reduce the percentage of seed germination. In a commercial germinator, using the paper-towel method of germination, fungi growing in the citrus pulp materially reduced the percentage of germination.

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BOOK REVIEW

ORGANIC INSECTICIDES, THEIR CHEMISTRY AND MODE OF ACTION. By Robert L. Metcalf. 392 pp. Interscience Publishers, Inc., New York. 1955. $8.50.

The present work is an outgrowth of the writer's monograph, "The Mode of Action of Organic Insecticides," published in 1948 as Review No. 1 of the Chemical-Biological Coordination Center, National Research Council, Washington, D. C.

This book presents in condensed form the most important information on the relation of the structure of organic insecticides to toxicity, their metabolism in plants and animals and their toxic hazards to higher animals. The 14 chapters deal with nicotine, nornicotine and anabasine; rotenoids; pyrethroids; joint action of insecticides; organic thiocyanates; dinitrophenols; DDT; acaricides (Neotran, Ovotran, Sulfenone, Aramite, etc.); benzene hexachloride; cyclodiene insecticides (chlordane, heptachlor, aldrin, dieldrin, isodrin, endrin and toxaphene); organic phosphorus insecticides; carbamates; miscellaneous insecticides (phenothiazine, ryanodine, sabadilla, hellebore, etc.); and insecticide resistance.

There is no discussion of organic compounds used as insecticidal fumigants such as methyl bromide, HCN, chloropicrin and ethylene oxide, nor is any mention made of insect attractants and repellents.

The references have been carefully selected and include many 1954 publications. Moreover, it is refreshing to this reviewer to observe that the patent literature is recognized as containing information of value to those working with insecticides and that reference is made to American and foreign patents.

This is an excellent book and every insect physiologist and serious student of insecticides will want a copy.

R. C. ROARK

Control of Soil Insects Attacking Peanuts[1]

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Soil insects attack peanuts in the commercial growing region of Alabama each year. Damage to peanuts by soil insects was known as early as 1889 according to Luginbill & Ainslie (1917), who listed a record of lesser cornstalk borer, *Elasmopalpus lignosellus* (Zell), attacking peanuts in Georgia. Fink (1916) described damage to peanut pods attacked by southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber. However, such damage received little attention until recent years. Miller (1943), while describing the injury to peanut pods by a white grub, *Strigoderma arboricola* Fab., also stated that the southern corn rootworm was the most destructive soil insect pest of peanuts in Vigrinia. Long & Dogger (1953) reported damage to peanut pods by the elongate flea beetle, *Systena elongata* (F.). In laboratory tests conducted by Grayson & Poos (1947), 5 pounds of technical DDT per acre gave complete control of southern corn rootworm, and in field experiments soil applications of DDT at 25 and 100 pounds per acre gave corresponding reductions of 47 and 84 per cent in number of pods injured. In preliminary work by Howe & Miller (1954), demeton soil drenches were ineffective for control of southern corn rootworm attacking peanuts.

Research has been conducted in Alabama since 1947 to determine the principal species of insects causing damage to peanut pods, to determine the extent of damage, and to evaluate the effectiveness of certain insecticides for control of the pests. Summarized results of experiments for the period 1947 through 1953 are herein reported.

Procedure.—The plot layout varied from year to year depending on the nature of the experiment. In most of the experiments where insecticides were applied to the soil, the plots were 4 rows wide and 33.3 feet long. In other experiments where insecticides were applied to the foliage, the plots were 6 to 12 rows wide and usually extended the length of the field. Treatments were replicated 3 to 4 times in randomized blocks. Blocks of plots were separated by alleys 4 to 15 feet in width.

Insecticides were applied to the soil as dusts, emulsifiable concentrates, or granules. Applications of insecticides were usually made to the soil by hand. Insecticidal dusts were applied with a rotary hand-duster to the foliage of plants on small plots. On larger plots a tractor-mounted duster was used. Some experiments included ethylene dibromide, which was applied to the soil with a soil-fumigant injector.

Soil applications of insecticides were made either before planting or during midsummer when the peanuts began to peg down. Foliage applications of insecticides were made at various intervals throughout the growing season. The insecticides used, rates, and time of application are given in the tables in which the results of the experiments are presented.

The percentage of insect-damaged pods was determined after harvest in most of the experiments. In determining the percentage of damage, each pod of a 200-gram sample of peanuts was inspected. The pods were separated into damaged and undamaged categories. The damaged and undamaged pods were counted separately, and the percentage of damage was calculated. In certain experiments, soil insect damage to peanut pods was determined before harvest by counting the number of insect-damaged pods and the number of undamaged pods per vine on two vines per plot. Insect damage to peanut kernels was determined by shelling 100 grams of peanuts and inspecting each kernel for damage in some experiments. The peanuts remaining in the soil after harvest were picked up and insect damage to the pods and kernels was calculated. The percentage of damage was determined as previously described except that a 50-gram sample of peanuts was used. The smaller weight sample was used because the total weight of peanuts removed from the soil of some plots after harvest was small.

Two methods of harvesting were used to determine yield of peanuts. In early experiments on small plots, the peanuts were dug, picked green by hand, and weighed. In later experiments, the peanuts were dug, allowed to cure in the field under natural conditions, and removed from the vines with a mechanical picker. The inner rows of each plot were used for yield records. All yields were based on pounds of cured peanuts per acre at 7 per cent moisture and 0 per cent foreign material. Moisture content determinations were made either with a commercial moisture indicator or with an electric drier.

Results.—Several species of soil insects have been found feeding on peanut pods in Alabama. The more common species were: southern corn rootworm, lesser cornstalk borer, banded cucumber beetle larvae, *Diabrotica balteata* Lec., and a wireworm, *Conoderus sp.*

Effect of Foliage Dusts and Sprays on Insect Damage to Peanut Pods.—Data on insect damage to peanut pods following foliage application of insecticidal dusts and sprays in 1948, 1949, and 1953 are given in tables 1, 2, and 3, respectively. Soil insects attacked a higher percentage of runner peanuts on untreated plots than on several of the dusted plots in 1948 (Table 1). Six applications of 20 per cent toxaphene dust were effective in reducing damage to pods 14 per cent below the damage of the untreated check plots. Only slight differences existed between the percentages of damage to treated or untreated Spanish peanuts. In 1949, 10 per cent toxaphene or 2.5 per cent DDT, either with or without sulfur-copper, applied to the foliage of peanuts reduced the damage to pods (Table 2). It appeared that all insecticides were about equally effective. The southern corn rootworm and banded cucumber beetle larvae were the two most abundant species of soil insects causing damage in 1949.

Summarized results of soil insect damage to peanut pods and kernels following application of insecticidal sprays in 1953 are shown in table 3. Four or six applications of DDT or six applications of toxaphene to the foliage caused significant reductions in damage by soil insects to harvested peanut pods. Of the peanuts left in the soil at harvest, only the plots treated with six applications of toxaphene had significantly fewer pods damaged by

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Table 1.—Insect damage to peanut pods following application of insecticidal dusts to the foliage, Headland, Ala. 1948.

Treatments[a]	No. of Applications	Average Damage to Peanut Pods			
		Runners		Spanish	
		Per Cent	*Angle*	*Per Cent*	*Angle*
Untreated		44.5	41.80	24.3	29.41
20% toxaphene	6	30.2	33.22	21.2	27.31
20% toxaphene	4	38.9	38.36	28.6	32.25
10% toxaphene	4	37.9	38.00	27.5	31.41
2.5% DDT+sulfur-copper	4	32.0	34.35	17.2	24.11
5% DDT and 2.5% DDT	2 & 4	35.1	36.30	24.7	29.51
2.5% DDT	4	36.3	37.02	31.9	34.07
L.S.D. at 5% level			3.85		—
1% level			5.28		—

[a] Dusts applied at approximately 20–25 pounds per acre per application. Four replications of each treatment.

soil insects. DDT or toxaphene, six applications to the foliage, significantly reduced soil insect damage to harvested peanut kernels. There were not significant differences in the damage to kernels left in the soil at harvest. In general, the systemic insecticides failed to control soil insects. A much higher percentage of the damage by soil insects was to the pods than to the kernels. However, damage to the pods by soil insects may result in destruction by soil fungi.

Effect of Foliage and Soil Applications of Insecticides on Insect Damage and Peanut Yields.—Soil applications of BHC, DDT, toxaphene, and chlordane dusts were effective in reducing soil insect damage to peanut pods in 1947 (Table 4). However, there was no increase in the yield of peanuts on treated plots. It is possible that extent of damage was insufficient to affect the yield of peanuts, since most of the treated plots produced slightly lower yields than the untreated plots. *Diabrotica* larvae and wireworms, *Conoderus sp.*, caused considerable damage in 1947.

Results of an experiment conducted in 1950 on control of soil insects are given in table 5. Soil insecticides were applied in the drill prior to planting. All treated plots had significantly fewer damaged pods than the untreated plots. Soil applications of BHC and aldrin were significantly more effective in reducing percentage of damage than were foliage applications of DDT. BHC caused a slight reduction in the stand of peanuts and a significant

Table 2.—Insect damage to peanut pods following application of insecticidal dusts to the foliage, Headland, Ala. 1949.

Treatment[a]	Average Damage to Peanut Pods[b]			
	Expt. I	Expt. II	Expt. III	Expt. IV
	Per Cent	*Per Cent*	*Per Cent*	*Per Cent*
Untreated	8.1	14.1	26.1	11.0
2.5% DDT+sulfur-copper	5.2	8.4	14.3	2.2
2.5% DDT+sulfur	—	—	—	2.6
2.5% DDT	—	—	—	3.0
10% toxaphene+sulfur-copper	—	—	—	2.2
10% toxaphene+sulfur	1.3	5.2	10.6	2.9
10% toxaphene	—	—	—	1.4

[a] Experiments I and II received four dust applications; experiment III, five applications; and experiment IV, six applications (rain followed two of the applications).
[b] Three replications except in experiment IV, which had four replications.

Table 3.—Insect damage to peanut pods and kernels following applications of insecticidal sprays to the foliage and soil, Headland, Ala. 1953.

Treatment[a]	Number of Applications	Pounds per Acre	Average Damage[b] to Peanut Pods		Average Damage[b] to Peanut Kernels	
			Harvested	Left in Soil	Harvested	Left in Soil
Untreated	—	—	29.72	49.99	11.76	40.30
Schradan	4	1.0	33.19	49.98	10.66	42.18
Demeton (Soil)	1	1.0	29.98	44.97	9.54	37.11
Demeton	1	0.5	28.28	46.39	10.99	46.62
Demeton (Soil & Foliage)	1 & 4	1.0 & 0.5	30.01	44.90	9.74	35.53
Demeton	4	0.5	29.82	45.36	8.11	38.13
Demeton	2	0.5	30.73	50.42	11.32	35.41
Chlorthion	4	0.5	33.57	50.81	10.53	40.77
Meta-Systox	4	0.5	32.96	47.20	9.73	35.93
DDT	2	1.0	25.02	46.16	9.86	42.67
DDT	4	1.0	23.14	51.05	9.09	41.13
DDT	6	1.0	23.15	46.37	4.97	45.88
Toxaphene	2	2.0	26.02	46.97	9.08	45.11
Toxaphene	4	2.0	31.31	47.87	9.30	38.55
Toxaphene	6	2.0	18.22	41.50	6.92	41.63
L.S.D. at 5% level			6.51	5.41	2.80	—

[a] Except where otherwise indicated, all treatments were applied to the foliage. Four replications of each treatment.
[b] Expressed as angles of percentages.

reduction in the yields. The only significant increase in yield of peanuts was on plots receiving foliage applications of DDT. This increase in yield resulted primarily

Table 4.—Insect damage to peanut pods and yield of peanuts following applications of insecticides to the soil, Headland, Ala. 1947.

Treatment[a]	Pounds per Acre	Damaged Pods		Yield per Acre	Gain
		Per Cent	*Angle*	*Lb.*	*Lb.*
Untreated	—	14.5	22.40	1766	—
BHC, gamma isomer	2.5	8.7	16.98	1699	− 67
BHC, gamma isomer	10.0	8.2	16.63	1700	− 66
DDT	20.0	9.3	17.58	1749	− 17
Chlordane	20.0	7.6	15.90	1584	−182
Toxaphene	20.0	8.9	17.23	1820	54
L.S.D. at 5% level			2.66	—	
L.S.D. at 1% level			3.68	—	

[a] Insecticides applied to soil when peanuts began to peg down. Four replications of each treatment.

Table 5.—Insect damage to peanut pods and yield of peanuts following insecticidal treatment, Headland, Ala. 1950.

Treatment[a]	Pounds per Acre	Damaged Pods		Yield per Acre	Gain
		Per Cent	*Angle*	*Lb.*	*Lb.*
Untreated	—	10.6	18.99	1463	—
BHC, gamma isomer	1	2.7	9.55	1172	−291
BHC, gamma isomer	2	0.9	5.31	875	−586
Aldrin	2	3.0	9.97	1342	−121
BHC, g.+2.5% DDT	2+20	0.9	5.47	1004	−459
2.5% DDT	20	7.2	15.46	2054	591
L.S.D. at 5% level			2.48	272	

[a] All treatments (four replications) applied to the soil at planting except DDT, which was dusted on the foliage on July 12 and 24, August 4, 15, and 25.

Table 6.—Insect damage to peanut pods and yield of peanuts following insecticidal treatment, Headland, Ala. 1952.

Treatments[a]	Pounds per Acre	Per Cent Damaged Pods	Pounds Yield per Acre	Pounds Gain
Untreated	—	3.5	1314	—
Dieldrin emul. in soil	2	6.5	1098	−216
Aldrin emul. in soil	2	5.8	1074	−240
Heptachlor emul. in soil	2	7.0	1251	− 63
Dieldrin dust in soil	3	5.9	959	−355
Dieldrin dust in soil+toxaphene dust on foliage	3 & 4	3.3	1791	477
Toxaphene dust on foliage	4	3.5	1906	592
DDT dust on foliage	2	4.1	1719	405
Dieldrin dust on foliage	0.4	1.5	1829	515
Parathion dust on foliage	0.4	1.3	1735	421
L.S.D. at 5% level			462	
L.S.D. at 1% level			623	

[a] Insecticides applied in soil on April 9 and to the foliage on August 8, 19, September 2 and 16, Four replications of each treatment.

from the control of leaf-feeding insects.

In 1951, the season was dry and yields were extremely low. The percentage of insect damage to peanut pods was not determined, but the lesser cornstalk borer was observed to be abundant in the vines and to a lesser extent in the pods. Yields of peanuts were not increased by applications of aldrin, chlordane, DDT, dieldrin, or heptachlor applied with the fertilizer before planting.

The results of an experiment conducted in 1952 where soil treatments of insecticides were compared with foliage treatments or a combination of soil and foliage treatments are given in table 6. Soil insect damage to peanut pods was light during 1952. Only slight differences existed between the percentages of damaged pods in treated and untreated plots. Where aldrin, dieldrin, or heptachlor emulsions or dieldrin dust was applied to the soil, there was a tendency toward reduced yields of peanuts. However, the differences were not significant. Plots receiving a soil application of dieldrin dust plus four foliage applications of toxaphene dust produced 477 more pounds of peanuts per acre than the untreated plots. Also, dieldrin, DDT, parathion, and toxaphene dusts applied to the foliage caused substantial increases in yield. The increase on dusted plots resulted largely from control of leaf-feeding insects.

Effect of Granulated Insecticidal Formulations on Soil Insects and Yield of Peanuts. More extensive work was conducted in 1953 than in any previous year on control of soil insects attacking peanuts. Experiments with granulated insecticides were conducted on sandy soils and on clay soils. Dusts were also used for comparison. The lesser cornstalk borer appeared to be the most important species causing damage. The results of these experiments are presented in tables 7 and 8.

More than twice as much damage occurred to peanut pods at Headland in sandy soil than at Troy in clay soil (Table 7). Plots receiving soil applications of aldrin dust, aldrin granules, lindane granules, toxaphene granules, or DDT dust had significantly fewer damaged pods than the untreated checks at Troy. No significant differences between treatments were recorded from pods at harvest or from those left on the ground. Before harvest at Headland on sandy soil, plots receiving soil applications of dieldrin, aldrin, lindane, and toxaphene, respectively, had significantly fewer damaged peanut pods than the check plots. After the peanuts were harvested with a mechanical picker, there were no significant differences in the percentages of damage to pods. In general, the percentage of damaged pods recovered from the soil was much greater than from pods picked by mechanical picker at harvest. Many of the pods damaged by insects before harvest were lost in the soil during harvesting operations.

The damage to peanut kernels was relatively light in comparison with damage to pods (Table 7). Damage to peanut pods grown in untreated clay soil averaged 10.5 per cent and damage in sandy soil averaged 20.4 per cent whereas damage to the kernels from the same plots was only 1.6 and 4.3 per cent, respectively. There were no significant differences between the percentages of damage to peanut kernels on treated or untreated peanuts grown in clay or sandy soils.

Yields of peanuts from the experiments on clay and sandy soils are shown in table 8. Yield differences between treatments were not significant on clay soil. Plots receiving soil applications of aldrin, dieldrin, and toxaphene granules at pegging-down time produced significantly more peanuts than the untreated checks on sandy soils at

Table 7.—Soil insect damage to peanut pods and kernels before harvest, on the vines at harvest, and left in the soil following insecticidal treatments, Headland and Troy, Ala. 1953.

Treatment[a]	Pounds per Acre	Average Damage Expressed as Angles of Percentages											
		Troy—Clay Soil						Headland—Sandy Soil					
		Before Harvest		At Harvest		Left in Soil		Before Harvest		At Harvest		Left in Soil	
		Pods	Kernels	Pods	Kernels	Pods	Kernels	Pods	Kernels	Pods	Kernels	Pods	Kernels
Untreated	—	17.1%	—	18.30	7.01	31.26	17.20	39.94	—	26.71	11.74	54.78	33.37
Aldrin granules	2	11.79	—	13.80	7.71	29.88	22.43	27.02	—	26.39	9.29	45.73	35.44
Aldrin dust	2	12.66	—	13.93	6.52	33.19	26.74	29.77	—	24.04	9.18	42.12	33.57
Dieldrin granules	2	14.18	—	15.24	5.74	28.11	16.34	23.00	—	21.54	8.44	41.66	35.28
Dieldrin dust	2	13.59	—	17.78	7.26	28.43	16.96	—	—	—	—	—	—
Heptachlor granules	2	15.48	—	17.77	8.01	35.76	20.97	35.68	—	27.22	9.71	52.02	38.13
Heptachlor dust	2	16.50	—	19.91	3.27	33.97	18.82	—	—	—	—	—	—
Lindane granules	2	12.43	—	14.83	7.55	31.64	21.17	30.29	—	25.76	8.25	46.43	33.69
Lindane dust	2	15.17	—	18.35	4.18	36.49	21.62	—	—	—	—	—	—
Toxaphene granules	6	11.14	—	16.49	2.76	29.90	16.49	34.91	—	26.33	9.51	50.33	35.89
DDT dust	4	12.18	—	15.26	3.27	30.63	18.31	—	—	—	—	—	—
Chlordane granules	5	13.95	—	19.70	6.97	27.87	12.52	—	—	—	—	—	—
L.S.D. at 5% level		3.72	—	N.S.	N.S.	N.S.	N.S.	7.07	—	N.S.	N.S.	10.51	N.S.

[a] Dusts applied to soil April 9; granulated materials applied July 7 and 8. Four replications of each treatment.

Table 8.—Yield of peanuts following application of insecticides to the soil, Headland and Troy, Ala. 1953.

Treatment[a]	Pounds per Acre	Average Yield per Acre (Pounds)							
		Troy—Clay Soil				Headland—Sandy Soil			
		Left in Soil	Gain	Normal Harvest	Gain	Left in Soil	Gain	Normal Harvest	Gain
Untreated	—	119	—	1061	—	266	—	1189	—
Aldrin granules	2	85	−34	1125	64	164	−102	1410	221
Aldrin dust	2	123	4	1148	87	191	−75	1301	112
Dieldrin granules	2	110	− 9	1224	163	84	−182	1631	442
Dieldrin dust	2	164	45	1110	49				
Heptachlor granules	2	104	−15	1008	−53	187	− 79	1248	59
Heptachlor dust	2	117	− 2	1106	45				
Lindane granules	2	92	−27	1177	116	114	−152	1337	148
Lindane dust	2	95	−24	1125	64				
Toxaphene granules	6	95	−24	1241	180	150	−116	1478	289
DDT dust	4	94	−25	1279	218				
Chlordane granules	5	83	−36	1166	105				
L.S.D. at 5% level		N.S.		N.S.	—	65	—	221	—
L.S.D. at 1% level						88		302	

[a] Dusts applied to soil April 9; granulated materials applied July 7 and 8. Four replications of each treatment.

Headland. Although relatively few peanuts were left in the soil, all treated plots on sandy soils had significantly fewer peanuts left in the soil than the untreated checks.

SUMMARY.—Replicated experiments were conducted in Alabama from 1947 through 1953 to determine the species of insects causing damage to peanut pods, to determine the extent of damage, and to evaluate the effectiveness of insecticides for control of the pests. The larvae of the southern corn rootworm (*Diabrotica undecimpunctata howardi*), the banded cucumber beetle (*D. balteata*), the lesser cornstalk borer (*Elasmopalpus lignosellus*), and a wireworm (*Conoderus sp.*) were the principal species of insects causing subterranean damage to peanuts.

Over the 7-year period, the percentage of damaged pods on untreated plots ranged from 3.5 to 44.5 per cent. The percentage of damage to peanut pods was much higher than to the kernels within the pods. Many of the peanut pods damaged by soil insects were lost in the soil during harvesting operations.

Four to six applications of 2.5 per cent DDT or 10 per cent toxaphene dusts for control of leaf-feeding insects were helpful in reducing damage by soil insects. Foliage applications of demeton, Meta-Systox, schradan, or Chlorthion were not effective in reducing damage to peanut pods.

Soil applications of DDT, BHC, chlordane, toxaphene, and aldrin dusts applied in the drill prior to planting reduced the damage to peanut pods by soil insects, but did not cause the yield of peanuts to be increased significantly. In one experiment, the yield of peanuts on BHC-treated plots was significantly lower than the yield of the untreated check plots. Soil applications of aldrin, dieldrin, and heptachlor emulsions in the drill prior to planting did not reduce the damage to peanut pods or increase the yield of peanuts in 1952.

In 1953, insect damage was reduced and the yield of peanuts was increased significantly above that of the checks where aldrin, dieldrin, or toxaphene granules were applied to the surface of the soil when the plants began to peg down. Aldrin and dieldrin were used at the rate of 2 pounds technical material per acre and toxaphene at the rate of 6 pounds. Gains from treatments on sandy soil at Headland, Alabama, ranged from 289 to 442 pounds of cured peanuts per acre. Gains were lower on clay soil at Troy.

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Field Tests with Insecticides against Cotton Insects at Tallulah, La., in 1954[1]

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Various organic insecticides were tested at Tallulah, La., in 1954 for control of the boll weevil, *Anthonomus grandis* Boh., the bollworm, *Heliothis zea* (Boddie), other lepidopterous larvae, the cotton aphid, *Aphis gossypii* Glov., and the spider mites *Tetranychus telarius* (L.) and *desertorum* Banks.

Methods.—Five experiments were conducted in plots arranged in randomized blocks, with four replications of each treatment. Each plot was 12 rows wide and of sufficient length to contain about 0.1 acre. All infestation and yield records were made on the four middle rows.

Boll weevil-injured squares were determined by examining 100 squares, bollworm-injured bolls by examining 100 bolls in each plot. Counts of aphids and spider mites were made by recording the number on 100 square inches of leaf area, 1 square inch being examined per leaf near the center on the underside of the fourth leaf from the top of each of 100 plants.

In experiments 1, 2 3, and 5 all insecticides were applied with single-nozzle rotary hand dusters early in the morning, and in experiment 4 with a three-row sprayer when the plants were dry. In the spray applications one nozzle per row was used through August 6, and three nozzles per row thereafter.

The variety of cotton in all the experiments was D & PL 15 and was planted between April 18 and 22. Because of the lack of moisture during the growing season, much of the cotton quit early, thereby lowering the yield. The entire area was affected in experiments 1 and 2, about half the area in experiment 3, and parts of two blocks in experiment 5.

The boll weevil was the most important insect, but there was some climatic control of this pest. The bollworm was of lesser importance particularly in those experiments where the cotton quit early. Spider mite and aphid infestations were very low, although spider mites had started to build up in some plots late in the season. Fleahopper counts in late June and early July showed insufficient population to warrant control measures for this pest.

Results.—The results of these experiments are shown in tables 1 to 3.

In experiment 1 all treatments gave satisfactory control of the boll weevil. The spider mite population was lowest in the plots treated with mixtures containing sulfur and highest in the plots treated with DDT plus heptachlor. All the mixtures gave some control of the bollworm. There were no significant differences in the yields.

In experiment 2 all the materials gave satisfactory control of the boll weevil, but the infestation was significantly greater in the plots treated with BHC (6 per cent of gamma isomer) plus 10 per cent of DDT than in the plots treated with BHC (3 per cent of gamma isomer), 5 per cent of DDT, and 2 per cent of Aramite. The spider mite population was lowest in plots treated with mixtures containing an acaricide, particularly parathion or

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Table 1.—Results of experiments 1 and 2.

Treatment (Figures in Per Cent)	Pounds of Dust per Acre[a]	Per Cent of Weevil-Punctured Squares: Initial Count (July 15)	Per Cent of Weevil-Punctured Squares: Average of 5 Counts (July 20 to Aug. 9)	Mites per Square Inch, Average of 3 Counts (Aug. 3 to 23)	Per Cent of Bollworm-Injured Bolls, Average of 2 Counts (Aug. 9 and 19)	Pounds of Seed Cotton per Acre: Total	Pounds of Seed Cotton per Acre: Gain Over Check
Experiment 1							
Untreated check		48	36	0.64	6.5	720	
Dieldrin 1.5+DDT 5	13	45	19	.64	2.3	1090	370
Dieldrin 1.5+DDT 5+sulfur 40	11	53	20	.18	3.5	1060	340
Dieldrin 1.5+DDT 5+parathion 0.5	12	43	19	.53	1.8	800	80
Dieldrin 1.5+DDT 5+Aramite 2	13	56	20	.32	2.0	1080	360
Heptachlor 2.5+DDT 5	13	51	23	1.17	2.4	840	120
Heptachlor 2.5+DDT 5+sulfur 40	15	49	18	0.09	1.8	890	170
Aldrin 2.5+DDT 5+sulfur 40	10	55	24	.18	4.1	850	130
L.S.D. at 5% level			7	.45	2.7		
Experiment 2							
Untreated check		48	43	1.33	3.8	990	
BHC, gamma 3+DDT 5	13	45	18	1.29	2.0	990	0
BHC, gamma 3+DDT 5+sulfur 40	15	45	18	0.40	1.8	1020	30
BHC, gamma 3+DDT 5+parathion 1	11	40	20	.12	2.1	1250	260
BHC, gamma 3+DDT 5+Aramite 2	15	49	17	.14	1.9	980	−10
BHC, gamma 6+DDT 10	8	55	23	1.05	1.1	1260	270
L.S.D. at 5% level			6				

[a] Average of 7 applications made on July 16, 21, 26, 30, and August 4, 10, and 13.

Table 2.—Results of experiment 3.[a]

Gamma Content of BHC Concentrate (Per Cent)	Average Pounds of Dust per Acre Application	Per Cent of Weevil-Punctured Squares		Aphids per Square Inch (Aug. 27)	Mites per Square Inch, Average of 2 Counts (Aug. 4 and 27)	Per Cent of Bollworm-Injured Bolls, Average of 2 Counts (Aug. 13 and 27)	Pounds of Seed Cotton per Acre	
		Initial Count (July 7)	Average of 7 Counts (July 13 to Aug. 10)				Total	Gain Over Check
Untreated check	—	28	62	1.00	0.50	11	1180	—
12–14[be]	11	34	40	0.48	.66	8	1860	680
12–14[c]	14	30	34	.42	.32	10	1530	350
12–14[d]	14	27	36	.35	.23	6	1800	620
35–40[e]	14	35	38	.47	.20	5	1590	410
35–40[e]	12	25	44	.62	.36	7	1750	570
35–40 Ground[d]	14	30	42	.29	.20	11	1670	490
35–40 Impregnated[d]	14	27	40	.13	.09	8	1870	690
Lindane[e]	13	32	44	.46	.15	11	1960	780
L.S.D. at 5% level			8.4	.46				

[a] Dusts contained 5% of DDT, 40% of sulfur, and 3% of gamma isomer of BHC from concentrates of different gamma content (blended dusts unless otherwise indicated) applied on July 9, 14, 23, 28, and August 2, 6, 11, 16, and 20.
[b] No sulfur.
[c] Supplied by Niagara Chemical Division, Food Machinery and Chemical Corp.
[d] Supplied by Ethyl Corp.
[e] Supplied by Virginia-Carolina Chemical Corp.

Aramite. The bollworm injury was very low in all plots. The differences in yield were not significant.

In experiment 3 the plots were treated with mixtures containing 3 per cent of gamma isomer from BHC concentrates of different gamma content. All treatments gave some control of the boll weevil but not as good as was expected. The best control was obtained with two mixtures formulated with BHC of low gamma isomer content (12 to 14 per cent), and the poorest with two prepared with BHC of high gamma content (35 to 40 per cent and lindane). All mixtures containing sulfur gave lower mite populations than the check or the mixture without sulfur. All but one of the mixtures formulated with a BHC of 35 to 40 per cent gamma isomer gave significantly lower aphid populations than the check, but the impregnated mixture gave the lowest population. There was some bollworm injury in this experiment, but there were no significant differences between any of the

Table 3.—Results of experiments 4 and 5.

Treatment	Average Pounds Acre Application[a]	Per Cent of Weevil-Punctured Squares		Aphids per Square Inch (Aug. 24)	Mites per Square Inch, Average of 2 Counts (Aug. 4 and 24)	Per Cent of Bollworm-Injured Bolls, Average of 2 Counts (Aug. 13 and 27)	Pounds of Seed Cotton per Acre	
		Initial Count (July 7)	Average of 9 Counts (July 12 to Aug. 24)[b]				Total	Gain Over Check
(Figures in pounds per gallon)				Experiment 4 (Sprays)				
Untreated check	—	25	55	0.08	0.02	11.0	2040	
Am. Cyanamid 12008 4	0.25	33	52	.08	0	6.6	1980	−60
Am. Cyanamid 12009 4	.29	43	55	.02	.01	10.1	1940	−100
Dieldrin 1.5	.16	40	37	.08	.01	6.5	2500	460
Endrin 1.6	.21	35	31	.07	.04	3.8	2730	690
Dieldrin 1+endrin 1	0.1+0.1	35	35	.21	.07	5.1	2720	680
Heptachlor 2	.28	31	34	.03	.02	6.8	2440	400
L.S.D. at 5% level			11			4.5	436	
(Figures in per cent)				Experiment 5 (Dusts)				
Untreated check	—	35	36	0.91	0.44	8	2190	
Toxaphene 20+sulfur 40	17	35	22	.40	.08	6	2090	−100
Endrin 2[c]	15	36	19	.26	.25	4	2590	400
Endrin 2[d]	17	39	15	.49	.66	3	2880	690
Endrin 2+sulfur 40[d]	17	30	16	.24	.04	4	2970	780
Endrin 2+sulfur 40[de]	15	32	17	.22	.10	2	2540	350
Chlorthion 3	12	34	34	.06	.10	6	2690	500
Strobane 20	14	34	24	.97	.22	5	2700	510
L.S.D. at 5% level			7	.52	.37			

[a] Pounds of active ingredients in sprays and of diluted dust in dusts. Average of 10 spray applications, made on July 8, 13, 23, 28, and August 2, 6, 11, 16, 20, and 25. Dusts applied on July 9, 14, 23, 28, and August 2, 6, 11, 16, 20 and 25.
[b] In experiment 5, 8 counts July 13 to August 18.
[c] Supplied by Shell Chemical Corp.
[d] Supplied by Virginia-Carolina Chemical Corp. Formulated with sterilized tobacco diluent.
[e] Contained deactivated sulfur.

treatments. All treatments gave moderate increases in yield, but there were no significant differences between treatments. The greatest increase in yield was obtained in plots treated with a mixture containing lindane.

In experiment 4 all treatments except Am. Cyanamid 12008 and 12009 gave satisfactory control of the boll weevil. The mite and aphid populations were very low in this experiment. Endrin and dieldrin plus endrin gave good control of the bollworm, resulting in significantly lower boll injury than no treatment or Am. Cyanamid 12009. Dieldrin, heptachlor, and 12008 also gave some control of the bollworm. Significant increases in yield over the check, 12008, and 12009 were obtained with endrin, dieldrin, and dieldrin plus endrin, and significant increases over 12008 and 12009 were obtained with heptachlor.

In experiment 5 Chlorthion was the only dust that did not control the boll weevil. Plots treated with endrin formulated with an inert tobacco dust, alone and with sulfur, had significantly less boll weevil injury than those treated with Strobane. Chlorthion gave the best aphid control. The Strobane plot was the only one that had a higher aphid buildup than the check. All insecticides except the endrin formulated with inert tobacco dust alone gave good control of spider mites, but the best control was obtained with Chlorthion and the three mixtures containing sulfur. The plots dusted with endrin had the least bollworm injury. Small but insignificant increases in yield over the check were obtained in all the plots except those treated with toxaphene plus sulfur. Two of the plots receiving this treatment were in the area which quit early because of the dry weather. None of the other treatments were given to more than one plot in this area.

Insecticides Used to Control House Fly Larvae[1]

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There has been considerable interest in California for several years in the use and effectiveness of certain insecticides employed in the control of the house fly through killing the larval forms. Enquiries concerning larvicides have not been limited to the standard persistent and nonpersistent types, but have also been concerned with some of the older and newer control agents, and with some common household materials. The situations usually have been concerned with surface accumulations composed of various kinds of organic matter.

Various chemicals have been recommended as house fly larvicides by investigators throughout the world.

Among the chlorinated hydrocarbon insecticides, DDT has been mentioned most often (Anon. 1946; Cunningham & Madden *et al.* 1947; McDuffie *et al.* 1946; McGovran *et al.* 1944; McGovran & Piquett 1945; Olson & Dahms 1945; Simpson 1946; Tanada *et al.* 1950; Travis & Bohart 1946; Wolfenbarger & Hoffman 1944).

Chlordane has also been used (Baker & Schoof 1955; Coffey & Maier 1950; Quarterman & Mathis 1952).

Lindane (Quarterman & Mathis 1952) and BHC (Baker & Schoof 1955; Madden et al. 1947; McGovran & Piquett 1945; Quarterman & Mathis 1952) have also been tested or recommended.

Dieldrin (Baker & Schoof 1955; Quarterman & Mathis 1955) has also been utilized.

Aldrin has also been investigated for use as a fly larvicide (Baker & Schoof 1955).

Endrin (Baker & Schoof 1955; Cunningham & Eden 1955) and Diazinon (Baker & Schoof 1955; Cunningham & Eden 1955; Wingo 1954) were included in various investigations.

Recommendations concerning the use of a number of other types of chemicals are to be found in the recent literature: borax (Hogan 1940; McGovran & Piquett 1945; Olson & Dahms 1945; Wolfenbarger & Hoffman 1944), chloride of lime (Dolinskaya *et al.* 1945; Joshi & Dnyanskaya 1947), crude oil (Joshi & Dnyanskaya 1947), gas condensate (Simmons & Dove 1945), hexachlorethane (Ketchova 1946; Ostakenya & Shapiro 1948), lethane (Madden *et al.* 1947); malathion (Mayeux 1954); paradichlorobenzene (Manis *et al.* 1942; McDuffie *et al.* 1946; Quarterman & Mathis 1952), phenothiazine (Wolfenbarger & Hoffman 1944), piperonyl butoxide (Mitlin & Konecky 1955), sodium arsenite (Babione 1944; Hering 1945; Madden *et al.* 1947), tar oil (Anon. 1942), and thiourea (McGovran & Piquett 1945; Wolfenbarger & Hoffman 1944).

In addition, Madden *et al.* (1947) discuss a number of relatively ineffective materials applied to house fly larvae, as well as the effect of a number of insecticidal solvents.

Goulding *et al.* (1953) included dieldrin, heptachlor, chlordane, aldrin, BHC, sodium, arsenite, lead arsenite, Paris green, calcium arsenate, fuel oil, toxaphene, and creosote in control measures for the larvae of salt-marsh sand flies.

Experiments were carried out in the field during 1952, 1953, and 1954 on the control of larvae of the house fly, *Musca domestica* L., by means of most of the chemicals mentioned above; not included in the tests were gas condensate, piperonyl butoxide, and hexachlorothane. A few additional materials were also tried.

The results of these experiments are given here.

PROCEDURES.—Preliminary tests were conducted with several of the chlorinated hydrocarbon compounds as dusts, suspensions, and emulsions in order to determine the most reliable form of application. Only the water emulsion seemed to be worthy of continued use; this agrees with the conclusion of Tanada *et al.* (1950). Accordingly, compounds not water-soluble were applied as emulsions.

The sprays were dispensed by means of a 3-gallon cylinder sprayer, at the rate of 225 milliliters of liquid to a square foot of surface of accumulations of organic mat-

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ter at least 2 feet square. A medium spray was used. Concentrations (as weight/weight percentages) used were those generally recommended by manufacturers or investigators for the control of house flies. The above amount of spray was found to be a minimum needed to secure good penetration.

A pint sample of organic matter was taken from each pile to be sprayed in order to make a pre-spray larvae count and to determine the species of flies present. The larvae were counted and identified in the laboratory, placed in a growth medium, and kept in holding cages. The flies that emerged were removed every other day, killed, counted, and identified. The data on these flies was used to compare emergence data from sprayed flies. The medium was discarded after 2 months.

A 6-inch square cage (of 18-mesh screen) 2 feet high was sunk into the sprayed piles in order to trap emerging flies. When it was not possible to use this type of cage, because of continual use of the pile or because of composition or configuration, a sample was taken of as nearly the same area, taken to the laboratory, and kept in holding cages. This material was discarded after 2 months. The number of flies in the cages in the field and in the laboratory was recorded weekly. A comparison of the number of flies emerged from sprayed surfaces was made with the unsprayed samples and the percentage of control computed. Results from material in cages in the field and in the laboratory were similar.

The composition and structure of each sprayed mass was investigated whenever possible after the 2-month period.

Types of organic matter included chicken manure, cow manure, lawn clippings and leaf rakings, garbage piles, cannery wastes (including that in cattle feed lots), slaughter house waste, and rabbit manure.

The effectiveness of the insecticides in preventing reinfestations was not investigated. It was noted, however, that there was some protection from the same chemicals used by Baker & Schoof (1953) and a few other materials.

Excluding the situations used in preliminary tests, a total of 136 piles of organic matter were sprayed in these experiments. At least three situations were used for each concentration of spray. Each sprayed pile was checked weekly during the 2 months for evidence of emergence outside the caged area.

RESULTS.—Of the compounds tested, the following gave little or no control or were effective only at high percentages or were erratic in action: borax, chloride of lime, crude oil, pyrethrum, lethane, thanite, tar oil, fuel oil, creosote, dinitrophenol, paradichlorobenzene (and a commercial sanitizing agent "De Sol," containing at that time an unspecified amount of PDB), aramite, diesel oil, Tide (a commercial detergent), soap, zinc naphthenate (effective at 4%), piperonyl butoxide-pyrethrum mixture, pentachlorophenol, potassium thiocyanate, soap, allethrin, dinitrophenol (erratic), BHC, and methoxychlor.

In the above group, borax, as a solution, was a notable failure—it did not give a complete kill until a 35% solution was used; at lower percentages up to 30% there was almost no kill. Chloride of lime was also of little use as a larvicide; a 24% solution offered at best about 14% kill.

Table 1.—Destruction of larval house fly infestations by treatment with certain insecticides.

INSECTICIDE	AVERAGE PER CENT KILL AT INDICATED PER CENT CONCENTRATION					
	3	2	1	0.5	0.25	0.125
Aldrin		100	100	99	90	
Chlordane		100	100	100	96	
Copper naphthenate		100	100	96	90	
Diazinon			100	100	100	98
DDT			100	100	100	94
Dieldrin			100	100	100	99
Endrin			100	100	100	100
Heptachlor			100	100	100	100
Lindane			100	100	100	100
Malathion			100	100	97	81
Naphthalene		100	100	98	48	
Parathion			100	100	100	100
Phenothiazine			100	100	100	61
Sodium arsenite	100	94	55	38		
Sodium pentachlorophenate			100	100	92	81
TDE	100	100	74	68		
Tetrachlorophenate		100	100	98	57	
Thiourea		100	100	94	86	
Toxaphene			100	100	100	96

Dinitrocresol give very erratic results—100% control at 3%, 92 at 2%, 89 at 0.5%, 100 at 0.25%, and 86 at 0.125%. Methoxychlor killed 91% of the larvae at 4%.

The effectiveness of the other compounds is shown in table 1.

It can be seen from data in the table that complete kills of larvae were obtained with aldrin (1%), copper naphthenate (1%), chlordane (0.5%), dieldrin (0.25%), DDT (0.25%), Diazinon (0.25%), endrin (0.125%), heptachlor (0.125%), lindane (0.125%), malathion (0.5%), naphthalene (1%), phenothiazine (0.25%), parathion (0.125%), sodium pentachlorophenate (0.5%), sodium arsenite (3%), TDE (2%), tetrachlorophenol (1%), thiourea (1%), and toxaphene (0.25%). At these percentages resistance might develop.

It should be noted that some of the compounds gave good control at concentrations lower than those indicated, exceptions being naphthalene, sodium arsenite, TDE, tetrachlorophenol, and thiourea.

DISCUSSION.—The above results are from the caged areas in the field and material collected in the field and held in the laboratory. The weekly observations on the sprayed, uncaged areas revealed a close correlation with control in the caged portions. It might be thought that these control data are exceptional; nearly the same results, however, were obtained by Baker & Schoof (1955) for larvae control in animal carcasses and by Goulding *et al.* (1953) in sand fly control work.

Extended observations during preliminary investigations revealed several interesting factors that might influence the amount of kill in a treated organic mass. It was the consideration of certain of these factors that lead to the use of a larger volume of spray than used by Tanada *et al.* (1950) and most people applying a spray for control of fly larvae.

The amount of moisture in the pile is important to the penetration of the larvicide as well as the development of

the larvae. It is obvious that a mass of organic material having a hard surface will not allow a spray to penetrate to any degree; much of the liquid will either be soaked up or run off. Hadjinicolou & Hansens (1953) noted that only the outer 3 or 4 inches of large piles could be treated by sprays; this statement probably applies to a moderately moist situation. When the pile has a fairly dry outer surface, the spray may not penetrate more than $\frac{1}{4}$ inch. In such situations, the persistent-type insecticides have a definite advantage over such "one-shot" insecticides as sodium arsenite or phenothiazine, providing of course nothing is done to nullify the effectiveness of the more lasting larvicides.

It was found that several light sprayings totaling 225 milliliters per square foot gave optimum results with piles having a hard surface. It was also found that an organic mass having a more or less uniform moisture content of about five times the dry weight of the material provided for good penetration and dispersion without excessive run-off or drainage. This is a moist, but not wet, situation in which house fly larvae develop normally. The surface must be loose and porous, and the interior must not have impervious obstacles, in order that penetration be even and deep.

The careful dissection of several piles in which control had been poor (naphthalene, 0.5%, 0.125%; aldrin, 0.5%, 0.25%) revealed a central core of dry material in which there was an average of 10 larvae per pint of medium. While these larvae were not numerous they were above average in size and developed into adults that mated and laid viable eggs.

Duplication of this field situation in the laboratory showed that house fly larvae can live and develop in a growth medium such as manure or bran leavings that gradually become dry or had a dry central area originally. The cycle of development was longer than normal.

Impervious or relatively impervious material in the pile may offer enough protection beneath it to enable larvae to escape harm. Such material includes paper, cans, bottles, fowl and animal bodies, flat pieces of sheet metal, and shingles and other flat, wooden objects. Investigation of three piles of lawn clippings in which failure of control (malathion 0.25%, 0.125%; chlordane 0.25%) had taken place revealed a complete half-folded daily newspaper in two cases (11×16 inches in area, 42 thicknesses deep) and several cedar shingles (an area about 12×14 inches) in the other case. These piles averaged 4 feet in diameter by 3 feet in height. Viable larvae and pupae were found under the newspaper and shingles; none was found in other areas of the piles.

In piles of a compact nature, either wet or dry, the larvae tend to form passage ways in which they move from one spot to another. In penetration studies, it was noted that the spray would sometimes follow these channels downward, to collect at the bottom of the pile. Larvae in areas not near these routes were unharmed until their movement brought them in contact with the larvicide.

In the preliminary experiments, before the amount of spray was increased, it was noted that nearly all of the more effective larvicides caused larvae to move away from the sprayed areas. This movement usually took place about a half hour after spraying ceased; occasionally it took place within a few minutes (when the dosage was heavy; there was perhaps an irritating or fumigating effect). Specimens of these larvae were collected, taken to the laboratory, put in a growth medium, and held in cages for 1 month. Apparently normal adults emerged from the pupae that developed.

Briefly, then, the following factors influence the effectiveness of the larvicide: amount of moisture in the pile; presence of impervious, shielding or deflecting material; presence of irrigation channels; and the driving-out-effect of the chemicals involved. It may well be that the reported variability in action of fly larvicides can be attributed to some of these factors. Studies on increasing the penetration and spread of insecticides in masses of organic matter, as well as detailed studies on their composition and structures and on the ecology of the fly larvae therein are greatly needed in order to obtain more efficient fly control by means of larvicides.

A factor indirectly connected with chemical control of fly larvae is the state of knowledge of poultry men, farmers, householders, and other people on whose land flies find excellent developmental conditions. A surprising number have a very sketchy knowledge of the relationship between fly larvae found in piles of lawn clippings or manure and the adult insects; Smith (1954) found this lack of correlation among poultrymen of Santa Clara County, California. Where some idea of the value of chemical control did exist, it varied from a full realization of the need for the simultaneous use of sanitation to a rather simple, faithful reliance on such household items as soap and other detergents. The use of soap for example, is linked with the use to control aphids on such plants as roses. There was often also a lack of knowledge as to the correct methods of applying sprays and the meaning of dosages. This factor of course adds to the difficulty of securing effective chemical control of fly larvae; the deleterious effects can be overcome by the educational efforts of entomologists and sanitarians.

SUMMARY.—Based on field results obtained at a minimal concentration (at 0.125%), endrin, heptachlor, lindane, and parathion appear to be highly effective as a house fly larvicide. Nearly as effective (at 0.25%) are Diazinon, dieldrin, DDT, and phenothiazine. Next in effectiveness (at 0.5%) are chlordane, malathion, and sodium pentaphenate. Last in order of effectiveness are aldrin, copper naphthenate, tetrachlorophenol, thiourea (all at 1%), TDE (at 2%), and sodium arsenite (at 3%).

The following compounds were deemed to be worthless for use as a house fly larvicide: borax, chloride of lime, crude oil, pyrethrum, lethane, thanite, tar oil, fuel oil, creosote, dinitrophenol, paradichlorobenzene, soap, a synthetic detergent, Aramite, diesel oil, zinc naphthenate, piperonyl, butoxide-pyrethrum mixture, pentachlorophenol, potassium thiocynate, allethrin, dinitrophenol, BHC, and methoxychlor.

Such factors as the moisture content, presence of impervious matter, larval migration channels, and driving-out effect of some of the chemicals were shown to have a relation to the supposed ineffectiveness of some insecticides. More studies are needed on the powers of penetration of sprays into piles of organic matter such as manure and on the ecology of fly larvae in such situations.

The fund of knowledge concerning flies among those

who have a need to control fly larvae is also discussed. The amount of knowledge may be very sketchy and ill-applied and tends to lessen the effectiveness of the insecticides. An increased educational program concerning correct control methods should be emphasized by entomologists and sanitarians.

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Tests with Organic Phosphorus Insecticides for Fly Control[1]

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The advent of organic phosphorus insecticides of low mammalian toxicity has stimulated much interest in their use for house fly control. Several have been found to be excellent toxicants in sweetened baits. They have been especially useful where flies are resistant to chlorinated hydrocarbon insecticides. Since Thompson *et al.* reported in 1953 on the use of TEPP, several investigators have published on the efficiency of other less toxic organic phosphorus compounds. In 1954, Langford *et al.* published on the effectiveness of several. They reported outstanding success with malathion, Diazinon and Bayer L 13/59.

During 1952 and 1953 the writers, in unpublished work, found malathion and Diazinon to be unusually effective as wall sprays for the control of house flies. With the introduction of the new organic phosphorus insecticides Pirazinon, Am. Cyanamid 4124, and Chlorthion, the writers made studies in 1954 to determine their value as toxicant for wall sprays and for use in sweetened baits. At the same time additional studies were made with Diazinon, malathion and Bayer L 13/59 in an attempt to

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Table 1.—Residue studies showing knockdown and mortality in flies when exposed to wooden panels treated with organic phosphorus insecticides with and without sugar.[a]

Insecticide[b]	Percentage Flies Down After				
	2 Hrs.	4 Hrs.	2 Hrs.	4 Hrs.	20 Hrs.
	Panels 6 Weeks Old		Panels 13 Weeks Old		
Malathion—1%					
Emul.—Sugar	12	68	21	51	80
Emul.—No sugar	0	0	1	3	4
W. P.—Sugar	0	1	1	1	3
W. P.—No sugar	5	15	0	0	3
Chlorthion—1%					
W. P.—Sugar	0	0	0	1	9
W. P.—No sugar	0	2	0	0	2
Diazinon—1%					
Emul.—Sugar	41	62	4	5	34
Emul.—No sugar	10	30	1	9	59
W.P.—Sugar	4	48	0	0	12
W. P.—No sugar	1	1	0	0	1
Pirazinon—1%					
W. P.—Sugar	1	4	6	8	12
W. P.—No sugar	1	7	9	20	31
Am. Cyanamid 4124—1%					
Emul.—Sugar	21	44	47	72	89
Emul.—No sugar	0	0	0	0	14
Bayer L 13/59—1%					
Water Sol. Sugar	38	67	54	84	96
Water Sol.—No sugar	6	14	1	2	6
Check	0	0	0	0	0

[a] Sugared solutions contained 12% sugar.
[b] W.P. = Wettable Powder.
Emul. = Emulsion.

compare their toxicity with the new insecticides mentioned.

One hundred and forty-nine dairy barns or livestock housing units were used in making the studies. These included dairy barns, loafing sheds, horse barns and chicken houses. In initiating the work the insecticides were first tested under laboratory conditions. Those showing promise were then taken to the field for practical evaluation. Laboratory tests were also used in an attempt to appraise differences in toxicants that were difficult to appraise in field studies.

Wall Sprays.—Malathion, Diazinon, and Am. Cyanamid 4124 as sprays containing 0.5% actual insecticide were found to give good fly control when used in farm buildings as wall sprays. They were applied with a hydraulic sprayer operated at approximately 150 pounds pressure, and the walls and ceilings were sprayed to the point of runoff. Results showed that sprays prepared with either emulsions or wettable powders, with and without sugar, (20 lbs. to 100 gals.) gave excellent fly control. In studying conditions in sprayed barns no great differences could be pinpointed between sugared and non-sugared sprays. Likewise there were no outstanding differences between sprays prepared from emulsions and those prepared from wettable powders. Trends, however, indicated that residues from emulsions held flies under control slightly longer, perhaps due to less "flaking off" of the insecticide. The period of time in which a given insecticide gave protection varied from barn to barn. Fly control in barns sprayed with Diazinon lasted considerably longer than in those sprayed with malathion. Diazinon gave protection for periods ranging from 30 to 60 days with an average of 40 days. Malathion gave protection for from 20 to 30 days with an average of 23 days. Am. Cyanamid 4124 was found to be equal, but not superior, to malathion in its lasting qualities.

Laboratory studies on residues were made by dipping a series of 5-inch square wooden panels into a 12% sugar solution containing 1% insecticide, and another series into a water solution of 1% insecticide without sugar. Results obtained in these laboratory studies were somewhat in conflict with those obtained in dairy barns, particularly as they relate to the lasting qualities of residues from Diazinon and 4124. The field observations indicated negligible differences between sugared and non-sugared sprays. The laboratory studies, table 1, indicated that sugar, 12% solution, definitely prolonged the residual life of the emulsified and water soluble insecticides. Laboratory studies also indicated that 4124 with sugar had residual life equal to or possibly greater than either Diazinon or malathion. Table 1 shows that with 4124 89% of the flies were down after 20 hours when exposed to 13-week old panels as compared with 80% for malathion and 34% for Diazinon. With sugar solutions that had aged for 17 weeks (Table 2), malathion, Diazinon, Chlorthion, 4124, and Bayer L 13/59 all retained a high degree of toxicity. Pirazinon showed a greater degree of deterioration.

Both the panel and aging tests, tables 1 and 2 indicated that Bayer L 13/59 with sugar should make an excellent wall spray. Late in the season one barn was sprayed with a solution containing 0.8% insecticide and

Table 2.—Results obtained with 17-week-old bait solutions for killing flies.

Insecticide[a]	Per Cent Knockdown After Intervals Indicated				
	1 Hr.	2 Hrs.	3 Hrs.	4 Hrs.	20 Hrs.
Malathion—1%					
Emulsion	7	63	75	85	98
Wettable Powder	9	58	78	86	99
Diazinon—1%					
Emulsion	16	52	94	100	100
Bayer L 13/59—1%					
Water Soluble	31	85	94	100	100
Am. Cyanamid 4124—1%					
Emulsion	18	61	84	93	100
Chlorthion—1%					
Wettable Powder	0	1	14	40	98
Pirazinon—1%					
Wettable Powder	4	5	9	12	64
Check	0	0	0	0	0

[a] Baits prepared as 12.5% sugar solutions.

6% sugar. The results were disappointing when compared with the results obtained in the panel and aging tests under laboratory conditions. The immediate effect from the spray was good. Flies were practically eliminated from the barn for a period of 3 days, but after that they increased rapidly. After one week the barn was dropped as an experiment and treated with another insecticide.

SWEETENED BAITS.—Malathion, Diazinon, Pirazinon, Chlorthion, Am. Cyanamid 4124, and Bayer L 13/59 were tested extensively in sweetened baits. Laboratory studies showed all were effective in wet baits (12% sugar) in which the insecticide content was varied from 0.25% to 1%. Baits with 1% toxicant gave from 90 to 100% kill in a 2-hour feeding period. With few exceptions flies, under laboratory conditions, fed on all baits promptly. Baits prepared with Bayer L 13/59 appeared to be more attractive than others. Under laboratory conditions flies fed more readily on this bait than on sugared water when the insecticide content was held to 1% or less. There was evidence that flies were repelled at initial feeding by 4124, however, the knockdown was very fast. Close observations indicated that mortality was not always due to feeding on the 4124 bait. There was strong evidence that vapors from the 4124 were responsible for some mortality.

Both wet and dry baits were tested extensively under field conditions. The wet baits were standardized so that each gallon of bait (water) contained 12% granulated sugar and 0.4% toxicant. Both wettable powders and emulsions were used in preparing the wet baits.

Wet baits were applied in barns and buildings with either a sprinkling can or a compressed air sprayer. In barns with concrete floors the sprinkling can was found to be effective in making bait applications. Where concrete floors were not available, spraying stanchion pipes, support beams, door jambs and other areas where flies normally rest, was an effective way of applying bait. The amount of bait used varied from barn to barn, depending upon its size. Two to three gallons of bait were normally used in treating the average 40 to 50-stall dairy barn. Dry baits were prepared by mixing granulated sugar and insecticide. These were formulated so that the finished bait carried 1% toxicant. They were applied to concrete floors by hand or shaker at the rate of from 2 to 3 pounds to the average 40 to 50-stall dairy barn. Two commercial dry baits were also tested. These consisted of ground sea shells made attractive to flies, and coated with 1% of malathion by weight.

All of the above toxicants; namely, malathion, Diazinon, Pirazinon, Chlorthion, Am. Cyanamid 4124, and Bayer L 13/59 when used regularly at 3- to 6-day intervals, preferably twice a week, were found efficient for keeping flies under control in farm barns. There were apparent minor differences between wet and dry baits, but differences between toxicants, if they existed, were difficult to observe. Wet baits usually gave a faster knockdown, and on the basis of observed trends tended to give slightly longer protection. The latter was particularly obvious when the wet baits were sprayed on stanchion pipes, support beams, window sills, and door jambs. The type of cleaning or sanitation followed by dairymen in Maryland undoubtedly was responsible for the poor showing of dry baits. The daily sweeping, hosing and liming that most dairymen practice appeared to reduce the effectiveness of dry baits more than wet baits. The period in which fly control could be obtained, with the several sweetened baits under test, was variable. When different toxicants were used in a series of barns, there was often great differences in the periods in which effective fly control was obtained. However, the pattern of these differences was never constant. Likewise, it was found that any toxicant used in the above manner showed differences that were often as great as any encountered between toxicants. With malathion as a wet bait the periods in which flies were practically eliminated from barns and control could be considered effective ranged from 2 to 15 days with a mean average of 7.5 days. The other toxicants gave results with variations that equalled or fell close to the above range. The mean average for others were: Chlorthion, 7.8 days; Am. Cyanamid 4124, 8.5 days; Diazinon, 7.5 days; and Pirazinon 7.6 days. Bayer L 13/59 held flies under control for from 3 to 5 days.

The work discussed above was supplemented with limited studies on wet baits prepared with one-half the above amounts of toxicant (0.2%) and sugar (6%), as well as baits prepared with twice as much (toxicant 0.8% and sugar 24%). The baits with the low toxicant and sugar content gave excellent results for period of 1 and 2 days, but for effective control it was necessary to use them daily. Trends indicated that the baits with the high toxicant and sugar content were superior to the standardized baits in that the minimum periods of protection were usually extended for from 1 to 3 days. But in most instances flies began to increase to such an extent after 7 to 8 days that additional treatments were needed. Thus, the average periods of protection for the baits with the high sugar and toxicant content fell close to those given by the standardized baits.

EFFECT OF AGE ON BAIT TOXICITY.—There is a tendency on the part of some farm people to prepare baits in quantities in excess of their immediate need. These are stored and used at a later date. The question of the effectiveness of these baits often arises. Baits prepared from malathion, Diazinon, and Bayer L 13/59 have consistently given good results after standing from 7 to 10 days. But to obtain more information a study was made on the toxicity of aging baits. Baits were prepared with 12% sugar and 1% insecticide and tested after a storage period of 17 weeks. Feeding tests (Table 2) showed that wet baits prepared with the organic phosphorus insecticides malathion, Diazinon, Chlorthion, Bayer L 13/59, Am. Cyanamid 4124, and Pirazinon deteriorated on standing. Seventeen-week-old baits gave a much slower knockdown than fresh baits, but with the exception of Pirazinon, all showed an unusually high degree of toxicity after a feeding period of 20 hours. A malathion bait that had been bottled and stored for 14 months was tested. After 1 hour 15% of the test flies were dead. After 4 hours, 77% were dead. This bait developed a very strong odor like that of H_2S, but this did not seem to have any deterrent action on the feeding flies. They fed readily but the killing action was obviously slow.

CONCLUSIONS.—Under conditions encountered in Maryland, and without consideration for such problems as

safety for animals and milk contamination, the organic phosphorus insecticides malathion, Diazinon, Am. Cyanamid 4124, Chlorthion, Pirazinon, and Bayer L 13/59 were found efficient for controlling house flies. All gave satisfactory kills when used in sweetened baits. Malathion, Diazinon, and Am. Cyanamid 4124 were found to be efficient as wall sprays either with or without sugar. Laboratory tests with treated wooden panels and aging sugar solutions indicated that others such as Bayer L 13/59, Chlorthion, and Pirazinon had possibilities as wall sprays.

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A Method of Sampling for the Drop Size of Aerial Spray Deposits[1]

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This paper reports a sampling method for estimating the atomization of a spray deposit and the reliability of that estimate as expressed by its confidence limits. The spray deposits studied were obtained by aerial applications from an altitude of about 50 feet over open ground.

The drops in aerial spray deposits are to a great extent stratified in size according to their perpendicular distance from the flight line. Drop size variation parallel to the flight line is by comparison unimportant. Therefore, an adequate record of the spray deposit of a flight should include an intensive sampling of the entire swath width by collecting drops at frequent intervals along a line perpendicular to the flight line.

To obtain a record of the drops an airplane, dispensing spray, was flown upwind (to minimize the sorting effect of crosswind) over a sample line placed at right angles to the flight line. This line consisted of oil-sensitive cards (Davis & Elliott 1953) placed at 5-foot intervals. On these cards oil-spray drops produce circular spots whose diameters are roughly proportional to the diameters of the spray drops producing them. At present, drops as small as 40 microns diameter can be measured by this method. Drops smaller than this were unimportant in the spray deposits used in this study. To avoid border effects, a strip around the edge of each card was excluded from consideration; hence the actual area used was 3×5 inches per card.

The record on these cards is considered an adequate representation of the deposit of the flight. Therefore, results obtained from sampling this record are assumed to apply to the deposit on the entire swath. It should be clear however, that the population to be sampled is the drops (above 40 microns diameter) on the cards; therefore, statistical inferences reached as a result of the sampling apply only to this population.

A typical distribution of drop diameters in an aerial spray deposit is given in the first and second columns of table 1. A measurement of central tendency called the mass median diameter (hereafter referred to as mmd) is useful for characterizing this skewed type of distribution because it is less affected than a mean by the counting error associated with the small drops in the sample.

The mmd is determined by plotting diameter class against the corresponding cumulative per cent of total volume on logarithmic probability paper (e.g., columns 1 and 4, Table 1). The mmd is the diameter corresponding to 50 per cent volume, i.e., half the spray volume is of drops larger and half is of drops smaller than the mmd.

Table 1.—The distribution of drop diameters in an aerial spray deposit.

Drop Diameter Class (Microns)	Number of Drops	Per Cent of Total Volume	Cumulative Per Cent of Total Volume
47	252	0.2	0.2
62	576	0.8	1.0
78	252	0.7	1.7
94	324	1.5	3.2
109	216	1.7	4.9
125	144	1.6	6.5
141	192	3.1	9.6
156	96	2.1	11.7
172	132	3.9	15.6
188	168	6.4	22.1
203	48	2.3	24.4
219	96	5.8	30.3
234	98	7.3	37.6
250	62	5.6	43.2
266	40	4.4	47.6
281	40	5.2	52.8
297	26	4.0	56.7
312	44	7.8	64.6
328	17	3.4	68.0
344	18	4.3	72.3
359	22	5.9	78.2
375	11	3.4	81.6
391	11	3.8	85.4
406	3	1.2	86.6
422	8	3.5	90.1
438	3	1.5	91.6
453	3	1.6	93.2
469	2	1.2	94.4
484	2	1.3	95.7
500	0	0.0	95.7
516	3	2.4	98.1
531	1	0.9	99.0
547	0	0.0	99.0
562	1	1.0	100.0

The cards were treated as if they consisted of a population of mmd's, rather than of drops. This made it possible to use standard statistical formulas to determine the mean mmd and its statistical error. Accordingly, for each mmd or random sampling unit (rsu) an area was marked in the same randomly selected position on all cards. From the drop diameters measured on these areas an mmd was computed. To obtain a sample of mmd's the process was repeated by selecting other positions at random.

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The statistical basis of the scheme adopted may be explained as follows: (1) by considering the cards as strata of the total card area, and drawing the sample as described above, one has a systematic sample with a random start, in which the sample size, or number of rsu's, is equal to the number of random starts; or (2) the cards are sampled as a volume rather than as a series of strata or areas, in which the sequence of cards represents depth. There is much greater variation in drop diameter in this dimension than in the other two dimensions, the length and width of the cards; and since it is efficient sampling to include as much variation as possible in the rsu, it is extended through the entire series of cards.

Of course, the rsu or mmd does not have to extend over the series of cards. An mmd could be computed from an area on a single card, resulting in much more variation between mmd's, but a much larger number of them. However, the time required to compute an mmd makes it necessary to keep the number of mmd's or sample size small. The most representative small sample is obtained by determining each mmd from areas on every card, as described above.

The area per card on which drops are to be measured for an individual mmd determination can be calculated by either of two methods, which are referred to in this report as the constant area method and the variable area method. In the constant area method, a single area is drawn per card and all the drops measured therein. Unfortunately, when this area is made large enough to adequately sample the occasional very large drops, an unnecessarily large number of small drops also must be measured. These small drops, as mentioned earlier, make only a minor contribution to the total volume of spray deposited and thus have a minor influence on mmd (columns 2 and 3 of Table 1).

In the variable area method, the size of the area per card on which drops are measured is varied with the drop diameter so that large drops are sampled more intensively than small ones. The number of drops counted for each diameter class is then weighted by the reciprocal of the area used for that diameter class before computing the mmd.

Before drops are measured for an mmd determination, a value, Σn_i, is assigned to the total number of drops to be included in the sample. This value will depend upon the time available and the rate of measuring drops. For the constant area method, if ΣN_i is the total number of drops in the population, A is the area per card being sampled (3×5 inches) and a is the area per card on which drops are to be measured to obtain Σn_i, then

$$a = \Sigma n_i \frac{A}{\Sigma N_i}.$$

For the variable area method, the number of drops, n_i, to be measured in a diameter class, d_i, was made proportional to the per cent of the volume of the population of drops composed of drops of diameter d_i. From this, a formula was derived for a_i, the area per card on which n_i is to measured:

$$a_i = \Sigma n_i \frac{A}{\Sigma(N_i d_i^3)} d_i^3.$$

The quantity $\Sigma(N_i d_i^3)$ is the sum of the cube of each diameter class multiplied by the total number of drops of that diameter class in the population. Approximate values for ΣN_i and $\Sigma(N_i d_i^3)$ can be obtained by a brief sampling prior to the mmd sampling. These estimates of the population enable one to plan so that the number of drops actually counted when sampling for mmd roughly equals Σn_i.

The concepts described above were tested on a set of cards from each of four different spray flights, with the following specific objectives in mind.

1. To determine how many independent determinations of mmd were necessary to reduce the sampling error (confidence limits at the 95 per cent probability level expressed as a per cent of the mean mmd) to 10 per cent, the accuracy desired for the spray deposits used.

2. To compare the time involved in sampling drops by the constant area method with that involved in the variable area method.

The spray flights were made on four different dates with a Stearman biplane equipped with conventional boom sprayer dispensing an oil-base spray. For the first and third flights, henceforth referred to as C-1 and C-2, respectively, a coarse spray previously determined as roughly 300 microns mmd was used. For the second and fourth flights, referred to as F-1 and F-2, a fine spray of roughly 100 microns mmd was used. Delivery rates were so adjusted that the spots on the oil-sensitive cards did not overlap.

All spray flights were made shortly before sunset, when optimum weather conditions for deposition of drops prevailed. At 5-foot intervals in the sample line aluminum plates, to which were attached oil-sensitive cards, were placed on top of 3-foot stakes. After the passage of the plane an interval of at least 10 minutes was allowed for the spray to settle before the cards were collected. They were then allowed to dry 24 hours or more before further use.

In the first flight, C-1, a very small preliminary sample of the total card area was first taken for the purpose of determining a and a_i. For this an area of 0.25 sq. in. was delimited on every fourth card. The diameters of all spots in these areas were measured, using a binocular microscope with an ocular scale. Two persons, an observer and a recorder, made the measurements. Places were exchanged often enough to avoid fatigue in using the microscope and to minimize observer differences in measuring the spots. As each spot was measured, it was pencil-checked on the card so that it would not be remeasured.

The number of drops in each diameter class was totaled for all cards. This work, as well as all other counts and computations in the study, was either checked arithmetically or redone by a second person.

From this preliminary sample, values for $\Sigma N_i/A$ and $\Sigma(N_i d_i^3)/A$ were calculated. The number of drops to be counted for each mmd, Σn_i, was made as small as possible since it was judged more advantageous to make as many mmd determinations as possible than to measure large numbers of drops for an individual mmd. A value of 100 for Σn_i was used in computing a (constant area method). The minimum area used on the cards was a square 0.5 ×0.5 inch because the error in delimiting the area was considered too large on smaller areas. When the a required

was smaller than this, only cards at appropriate intervals were used. Thus, a used in C-1 was 0.25 sq. in. every third card. It was selected at random from the 45 sq. in. on the first three cards and then drawn in the same position on every third card thereafter. This procedure was repeated 6 times, making 6 independent determinations of the mmd.

In the variable area method, an a_i was computed for a Σn_i of 50. The a_i as computed from the formula was different for each diameter class, but since it was obviously impractical to mark a different area for each d_i a compromise was necessary, making d_i cover certain diameter class ranges as follows.

d_i Diameter Class Range (Microns)	a_i Area (Square Inches)	w_i Weight
47–125	0.25 every 18th card	36
141–219	0.25 every 6th card	12
234–297	0.25 every card	2
312 up	0.50 every card	1

The a_i was selected at random, in the same manner as described above for the constant area method. Six determinations were also made here. Thus, for flight C-1 there was a total of 12 determinations of mmd, 6 by each of the 2 methods. Time records were kept for the measurements and computation, which were done as described above for the preliminary sample.

For each method the mean and error (95 per cent probability level) and the mean time required *per determination* were computed. These are given in table 2. The number of mmd's necessary for a 10 per cent error was estimated by the standard statistical formula, $n = t^2 s_x^2 / \bar{x}^2 P^2$, in which n is the number of mmd's needed for the permissible error, P; and s_x^2 and $\bar{x}$ are the variance and mean, respectively, estimated from the sample of mmd's. The value of t depends upon the probability level (95%) and n-1. Results showed that 8 determinations involving 15.2 man-hours would be required to arrive at a 10 per cent error by using the constant area method, whereas only 4 determinations, or 10.8 man-hours, would be required by using the variable area method (Table 2).

In flight C-2 no preliminary sample was necessary, as the values of a and a_i computed for C-1 were deemed applicable to this flight. In the variable area method, however, a_i was modified slightly for the diameter class ranges, as seen below.

d_i (Microns)	a_i (Square Inches)	w_i
48–127	0.25 every 24th card	96
143–222	0.25 every 8th card	32
238–302	0.25 every card	4
317 up	1.00 every card	1

The constant area and variable area samples were taken in the same way as in C-1 with the exception that only four mmd determinations were made by the variable area method.

Results for this and subsequent flights, by each method, are shown in table 2. To arrive at a 10 per cent error, 17 determinations or 30.6 man-hours would be required by the constant area method, and 5 determinations or 13.5 man-hours by the variable area method.

Flights F-1 and F-2 with fine spray were treated in the same way as the coarse spray flights. In the constant area method, the F-1 value of a was 0.25 sq. in. on every 12th card. In the variable area method, the F-1 value of a_i was as follows.

d_i (Microns)	a_i (Square Inches)	w_i
48–111	0.25 every 16th card	64
127–143	0.25 every 4th card	16
159 up	1.00 every card	1

To obtain 10 per cent accuracy, 7.2 man-hours would be required for 9 determinations by the constant area method, and 4.5 man-hours for 3 determinations by the variable area method (Table 2).

In F-2, a was the same as in F-1, and a_i (variable area method) was only slightly modified, as follows.

d_i (Microns)	a_i (Square Inches)	w_i
48– 95	0.25 every 16th card	64
111–143	0.25 every 4th card	16
159 up	1.00 every card	1

Predicted time for 10 per cent error was 4.5 man-hours for 5 determinations by the constant area method and 7.8 man-hours for 3 determinations by the variable area method (Table 2).

Discussion.—The procedure of drawing a small sample of mmd's proved practical and can be applied to future drop size studies. For these four flights the predicted sample size, using the variable area method and selecting 50–150 drops per mmd, was 3 mmd's for the fine spray and 4 or 5 mmd's for the coarse spray. These figures cannot be arbitrarily applied in estimating the drop size of other spray deposits; however, they do give a general idea of the sample size required by the above method. The mean and error of a small number of mmd's can be computed in a few minutes. Therefore, when information is lacking on how many determinations of the mmd are necessary, time will be saved by computing the error after two or three of them have been made. From this the number of additional determinations needed, if any, can be calculated.

In all four flights the variable area method required more time per mmd for measuring and computation than the constant area method did, as shown in columns 6 and 7 of table 2. In the former method, time is lost in determining which drops on a given area are to be included in the count. Computation is also slower because of the weighting and because more diameter classes are involved. However, the sample of mmd's obtained is more representative, as indicated by the errors given in columns 2 and 3 of table 2. In three out of four flights this fact more than counterbalanced the additional time required per determination, making the variable area method the more

Table 2.—Determination of mmd[a] for two coarse and two fine spray deposits.

Flight No.	Mean MMD (Microns) and % Error		Mean Number of Drops per MMD		Time per MMD (Man-Hours)		Time for 10% Error (Man-Hours)	
	Constant Area	Variable Area	Constant Area	Variable Area	Constant Area	Variable Area	Constant Area	Variable Area
C-1	292.8±12.5	280.7± 6.5	89	65	1.9	2.7	15.2	10.8
C-2	312.5±20.4	298.5±11.5	105	84	1.8	2.7	30.6	13.5
F-1	90.3±13.4	92.5± 4.8	52	72	0.8	1.5	7.2	4.5
F-2	93.5± 7.2	98.5± 3.6	70	115	0.9	2.6	4.5	7.8

[a] Mass median diameter.

efficient (columns 8 and 9). In the last flight, however, the predicted time for a 10 per cent error was less for the constant area method.

The four flights tested here do not provide conclusive proof of the superiority of one method over the other. Several difficulties in comparing the two methods should be noted. One is the lack of control over the number of drops counted per mmd (columns 4 and 5, Table 2), which affects the time required per mmd. The number measured by the constant area method could be made more exact by a more thorough preliminary sampling, but it would be quite difficult to control it in the variable area method because the grouping of diameter classes, which is necessary from the practical point of view, affects the number of drops measured.

It is possible that the efficiency of one method may be improved more than that of the other through practice in measuring and computation. This study suggests that a variable area method of selecting drops may be advantageous for a spray with a wide range of drop sizes, but of lesser value or perhaps disadvantageous where the range is restricted. With either method, the time required for an mmd determination can be further reduced by simplifying the measuring process, short-cuts in computation, and improving the method of randomizing the sample of mmd's.

SUMMARY.—A method was devised for sampling drop records in determining the atomization of aerial spray deposits. It requires the selection at random of a small number of mass median diameter determinations and computation of the mean mmd and error of this sample. In turn, each determination involves measuring drops on an area on each of a number of cards placed at regular intervals across the spray swath. Two methods of selecting drops for an mmd determination were used. In one, the constant area method, a single area was marked on each card and all drops within the area were measured. In the other, the variable area method, the size of the area was varied approximately as the cube of the drop diameter, so that large drops were sampled more intensively than small ones. With respect to time required for drop measuring and computation of a mean mmd with a 10 per cent error, the variable area method proved superior in three out of four flights. Additional comparisons of the two methods will be continued in future studies of the atomization of aerial spray deposits.

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Chinch Bug Control on Lawns in Florida[1]

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Although chinch bugs are usually represented as being most destructive in the Mississippi, Ohio, and Missouri River Valleys, a severe and somewhat unique chinch bug problem has existed in Florida for many years. In Florida these insects have been troublesome almost solely on the popular St. Augustine lawn grass, *Stenotaphrum secundatum* (Walt.). St. Augustine grass lawns are subject to extremely damaging insect attack, and a chinch bug, *Blissus insularis* Barber, is the outstanding pest. Tremendous efforts are made to establish and maintain attractive landscapes in this state, especially in the tourist centers, and large sales of materials for use on lawns provide the "bread and butter" of a number of garden supply stores, pesticide formulating firms, and commercial spray operators.

In recent years, chinch bug control tests have been conducted by different workers in several areas of Florida. These have resulted in varying data and control suggestions (Kelsheimer 1953, 1954; Kuitert & Nutter 1952; Wolfenbarger 1953). In a state with such wide differences in latitude, types of soil, and kinds of turf, differences in control recommendations are not surprising. Further information on control is needed, and the tests described in this paper are the first steps in research directed at finding the best method of attaining chinch bug control and why some of the variations in control have appeared.

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[2] These tests were made possible through the cooperation of Kenneth Clark, G. E. John, and W. M. Armistead in Sarasota; J. W. Rogers in Jacksonville; and Sidney Kirkpatrick and Kenneth Anderson in Lauderdale-by-the-Sea. The author wishes to offer especial appreciation for the vital assistance given all through these tests by Mrs. Kerr.

Table 1.—Results[a] of chinch bug control tests on St. Augustine grass lawns in Florida.

Treatment	Pounds Actual Toxicant per Acre	10 Days After 2nd Application				Residual Effects Days After Last Application		
						Sarasota		Jacksonville
		Sarasota	Jacksonville	Lauderdale (1)	Lauderdale (2)	20 Days	48 Days	26 Days
Check		16.7	19.7	45.0	60.7	22.3	25.7	10.7
Parathion	1.5	0.3[b]	0.0[b]	5.0[b]	7.7[b]	0.7[b]	12.7[b]	7.3
DDT	10	0.7[b]	0.3[b]	7.0[b]	5.0[b]	0.0[b]	6.3[b]	0.0
Chlordane	10	3.3[b]	0.3[b]	41.3	108.3	18.7	35.0	1.3
Dieldrin	5	16.0	0.0[b]	33.7	23.3	47.0	58.7	1.7
Demeton	1	14.0	3.0[b]	—	—	25.0	61.3	6.0
Strobane	4	—	—	26.7	46.3	—	—	—

[a] Expressed in average number of chinch bugs per square foot.
[b] Significantly less than check at 5% level.

Several articles (Decker *et al.* 1953, Gannon & Decker 1955a, Gannon & Decker 1955b, Kearns *et al.* 1949, Walton 1947) have indicated that the order of toxicity of certain insecticides to chinch bugs in the Midwest is different from that observed in Florida. This is not particularly surprising since specialists in Hemiptera state that two species of insects are involved. The form found throughout Florida has been elevated to species status. In addition to morphological considerations there is the fact that *B. insularis* is not a pest of corn or other grains in Florida. There are indications of physiological differences. DDT is one of the most effective insecticides for chinch bug control in Florida, but for years DDT has been considered an inferior material for chinch bug control in the Midwest. The environments in which the Midwestern and Florida chinch bugs live are enormously different.

METHODS AND MATERIALS.—A standardized test was conducted on home lawns in some representative areas of the state in the summer of 1954. Plot size was 65 square feet, and each treatment was replicated three times. While chinch bugs have a patchy distribution in lawns, each block had a more or less uniform chinch bug distribution within it. Sometimes there were large differences in the density of the populations between blocks. Prior to every insecticidal application, the lawns were irrigated with about 1 inch of water. The insecticides were applied in the form of emulsion concentrates with a sprinkling can at the rate of 5 gallons per 100 square feet of lawn. Materials[3] and dosages used are shown in table 1.

The treatments were applied at 7 to 10 day intervals. Two applications were made except for the test in Sarasota, where three applications were made. A dosage series could not be included in these tests; but, since DDT and dieldrin have been the principal insecticides suggested for chinch bug control and since they had apparently failed under certain conditions for other research workers, these materials were given extra attention. One or two additional plots were set up at each location; on these dieldrin and DDT were tried at extremely heavy dosages, approaching those used by Wolfenbarger (1953) in the southernmost part of the state. If the dosages used in the test proper were not effective, the high levels would certainly have pointed out any toxic qualities if present and also they might have confirmed if such high dosages were needed under the conditions existing in some parts of Florida where control has been difficult. Dieldrin was applied at 25 pounds actual per acre and DDT at 100 pounds actual per acre. No follow-up applications were made at these levels.

The first test was started in Sarasota on April 6. The test in Jacksonville began on June 24. The two tests in Lauderdale-by-the-Sea started on September 9. Chinch bug counts were made 7 to 10 days after each insecticidal application by sampling 1 square foot of each plot. Later counts were made to observe residual effectiveness of the insecticides. A metal cylinder 1 square foot in cross-section was pushed a few inches into the turf and filled with water. The grass was agitated by hand and the bugs which floated to the surface during a 10-minute period were counted. It was not feasible to make more than one count per plot. This sample was deliberately taken where it was thought that the chinch bug population was densest. When these insects are present in St. Augustine grass, their location is obvious for they group together and feed at the margins of the damaged grass areas. Thus the samples were taken along the margins of damaged patches.

The method of maintenance of St. Augustine lawns appears to be important in the ease or difficulty of controlling this chinch bug. St. Augustine grass is quite versatile in its maintenance requirements. In some areas many lawns are maintained on a near starvation program. This results in a thin turf, with fibrous and unsucculent grass. It is relatively easy to control chinch bugs in such turf. In contrast, many lawns especially in the tourist centers, receive excessive water, top dressing, and fertilizer. These become very lush and succulent, dark green in color, and develop an extremely thick thatch which may be 6 to 10 inches deep. This appears to provide a very favorable habitat for chinch bugs and results in an inordinately difficult, three-dimensional problem of getting insecticidal penetration into the thatch. It has been assumed that large amounts of insecticide per unit area are required on these high maintenance

[3] Insecticides used in these experiments were supplied by the following manufacturers: California Spray Chemical Corporation, Chlordane, dieldrin, DDT, and parathion; Florida Agricultural Supply Corporation, parathion; Tobacco By-Products and Chemical Corporation, dieldrin; Chemagro Corporation demeton; B. F. Goodrich Chemical Company, Strobane.

lawns, but the tests described here were predicated on the idea that more moderate amounts of toxicants would do the job if they could be applied so that they would reach the insects.

The tests in Jacksonville were conducted on a very thin turf. At Sarasota the test was made on a sandy soil. The grass had not been top dressed regularly but the turf was deep, the soil much looser than the compacted and rocky artificially built land in Jacksonville, and the grass was lusher and greener. When the test was started in Sarasota, the number of chinch bugs in the test area was low, but they multiplied steadily and became extremely damaging in some plots. In the two test areas in Lauderdale-by-the-Sea, the thatch was exceptionally deep and the chinch bug population very high. Not only were these conditions the most extreme but the weather was more severe there. There were about 12¼ inches of rain during the 3-week testing period, the bulk of it falling in showers starting within 48 hours after each insecticidal application.

RESULTS.—In table 1 attention is directed to the count taken 10 days after the second application or 20 days from the start of each test. A count taken about 10 days after the first application shows just as significant reductions in chinch bug numbers. These first-count data are not shown or discussed since it has been felt that at least one follow-up application should be made to prevent a rapid resurgence in chinch bug numbers from the thousands of hatching nymphs. It was not possible to make observations on the effectiveness of one application versus two applications in the 1954 tests.

Parathion and DDT were effective at all locations. The high-level dosages of DDT, also applied at each location, did not give increased control. The data taken at Sarasota indicate that when parathion is soaked well down into the thatch it may have a considerable residual power. On the thinner turf at Jacksonville, significant control was not shown 26 days after the last application.

Chlordane did well on the thin turf at Jacksonville and against the light infestations early in the test at Sarasota, but did not give control under more extreme conditions as shown in the later Sarasota counts and at Lauderdale-by-the-Sea.

Except on the thin turf at Jacksonville, dieldrin gave consistently poor control. The high-level dosages of dieldrin also applied at each location did not provide control either.

Demeton was tried in the early tests, but it was evident that it was not effective and was dropped from further testing.

Strobane had shown good potential in preliminary trials similar in design to the tests reported here (Ritty 1954), and this material was used in the tests at Lauderdale-by-the-Sea. Under the extreme conditions of these two tests, Strobane gave less than a 50 per cent reduction in chinch bug numbers.

The data for parathion, DDT, chlordane, dieldrin, and check at each location were brought together in a combined analysis. There was a significant F for treatments, locations, treatments times location, and blocks within location. This gives statistical credence to some obvious observations. There was indeed significantly better chinch bug control at some locations than others. Also, it is evident some treatments had different effects at different locations. There were important differences between blocks at a given location, indicating that the differences in the size of the chinch bug population between blocks at the start of the test may affect the results of the treatments.

SUMMARY.—A standardized test for chinch bug control was conducted on lawns in several locations in Florida. Satisfactory control was obtained at all locations with DDT and parathion. Thickness and lushness of the turf seemed to be important in affecting the control obtained with the other insecticides. On a thin turf many materials apparently can give satisfactory control, but under extreme conditions some of the toxicants did not perform well.

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Research on the Use of Systemic Insecticides for the Control of Livestock Pests[1]

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The search for a systemic insecticide, toxic to the insect but nontoxic to the host, that does not cause a harmful residue has been almost continuous for the last three decades. The accomplishments were few until Hurd-Karrer & Poos (1936) discovered that wheat growing in soil containing soduim selenate was not attacked by the aphid, *Macrosiphum granarium* (Kby). This work stimulated entomologists to continue research in the field of systemic insecticides for the control of plant and animal insects. Research on systemics for plant insects has now reached the stage of practical success in the utilization of some of the phosphorus insecticides, but progress in the control of livestock insects has not yet been as rewarding.

The first success at internal administration of an insecticide to control a bloodsucking insect was attained by Knipling (1938), but that was not a case of true systemic control. Knipling rendered manure toxic to horn fly larvae by feeding phenothiazine to cattle. Some of the insecticide passed through the digestive tract unchanged to make the manure toxic. In thinking of systemic insecticides for animal treatment, one visualizes a chemical that when administered internally renders the animal toxic to an insect. This was first accomplished in 1943, when Lindquist *et al.* (1944) found that rabbits tolerated DDT or pyrethrum extract given orally in sufficient amount to render the blood toxic to bed bugs, *Cimex lectularius* L. and *hemipterus* F. Meillen (1946) reported that gamma BHC fed to rabbits made their blood toxic to insects. In 1946 and 1947 E. F. Knipling in Washington, D. C., and A. W. Lindquist in Corvallis, Ore., found that 2-pivalyl-1,3-indiandone given orally to dogs killed their lice, *Linognathus piliferus* (Burm.). Knipling *et al.* (1948) found that 2-pivalyl-1,3-indandione given to rabbits orally, subcutaneously, intramuscularly, or intravenously rendered the animals toxic to body lice, *Pediculus humanus corporis* Deg.

Early Tests Against Cattle Grubs.—When the Kerrvile, Texas, laboratory was established in 1946, the study of systemic insecticides for animal treatment was made a full-time project for two entomologists. The first experiments were conducted by Barrett & Wells (1948). They developed techniques for conducting *in vitro* and *in vivo* tests against the common cattle grub, *Hypoderma lineatum* (DeVill.). In the *in vitro* tests they mixed insecticides with bovine serum and observed their effects on larvae removed from the gullets of slaughtered cattle. If a chemical was larvicidal *in vitro*, it was further tested by administration to white mice that were artificially infested with cattle grubs taken from gullets. That work was continued through 1951 and hundreds of chemicals were tested without special success. Most of the materials toxic to the larvae by the *in vitro* test seemed also to be toxic to the mice.

Special emphasis was given to testing against cattle grubs for three reasons. In the United States cattle grubs are the most damaging insect parasites of livestock, causing losses estimated by Haeussler (1952) to average $160 million per year during the period 1940–44. The second reason is that the larvae spend so many months within the body of the host that they seem particularly susceptible to control by a systemic insecticide. The third reason is that present control measures for cattle grubs are far from satisfactory. The only method so far devised for controlling grubs in range cattle is back treatment with a rotenone insecticide. It is believed that a systemic insecticide offers the only hope of obtaining adequate control of these grubs within the United States.

In the course of the preliminary testing against cattle grubs, lindane was tried at Kerrvile without success. De Toledo & Saur (1950) reported that BHC administered to cattle infested with the human bot fly, *Dermatobia hominis* (L., Jr.), functioned as a systemic insecticide. Since this insect is related to the cattle grub, it was believed that a material effective against *Dermatobia* might control *Hypoderma*.

Screening Tests With Screw-worms.—Because attempts against first-instar cattle grubs had been generally unsuccessful, as a new approach the screw-worm, *Callitroga hominivorax* (Cqrl.), was selected as a screening insect. Testing against screw-worm larvae offered many advantages. The insects are easily reared in the laboratory, and a thriving colony was already being maintained at Kerrville for other research purposes. As the larvae feed in wounds of any warm-blooded animal, they can be used in almost any host desired. Since they complete their growth in 6 days and their entire life cycle in about 3 weeks, results are quickly evaluated. Finally, screw-worm control by means of a systemic insecticide is in itself a valid objective, since the insect does millions of dollars worth of damage each year in the Southern States and the wound treatments so far devised are not suited to all types of screw-worm cases. The preliminary experiments with screw-worms have been reported (McGregor *et al.* 1954, 1955).

For the first screening tests guinea pigs were given hip wounds and each pig was infested with approximately 50 newly hatched screw-worm larvae. When the insects had been in the wounds for 24 hours and were well established and growing viorously, a test insecticide was given the host animal. All the well-known chlorinated hydrocarbon insecticides were tested. Each material was dissolved in Tween 20 (polyoxyethylene sorbitan monolaurate) at 5% concentration and was administered subcutaneously in a sufficient amount to give a dose of 50 mg./kg. If it killed the host, it was tried at successively lower concentrations until the guinea pig was not affected.

In the early work with chlorinated hydrocarbon insecticides more elaborate studies were made than are now followed in routine screening. When DDT, toxaphene, and chlordane were tested, the larvae grew normally when guinea pigs were given a dose of 50 mg./kg. The insects

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were allowed to complete their development in the host, crawl out, pupate, and emerge as adults, The flies were maintained in cages for 7 days, when the females were forced to oviposit so that observations could be made upon their fertility.

With lindane, dieldrin, and aldrin it was found that appropriate doses killed the larvae without harming the host. The duration of effectiveness of a treatment was checked by periodically reinfesting a wound with newly hatched larvae. If it was found that larvae could become established in a wound, observations were continued to note whether the insects grew normally. Still later wounds were infested to observe whether there was undue mortality in the pupal stage or whether fertility of the adults was affected. It was noted that after a treatment a guinea pig would remain toxic to larvae so that they could not establish in the wound for several days. Larvae introduced after that would grow, but they died in the pupal stage. If the wound was infested still later, some larvae would even pass through the pupal stage and emerge, but the flies would show symptoms of insecticide poisoning and soon die. When infested still later, normal flies were reared and no effect on the fertility of the adults was noted.

The screw-worm testing program has now expanded so much that it is too cumbersome to test insecticides for all these degrees of effectiveness, and we drop consideration of a chemical if the larvae continue to grow normally after guinea pigs have been given a maximum dose. In checking on its residual effectiveness we stop testing when we find that newly hatched larvae can establish and grow in a wound. By adopting such rules we have been able to subject many more chemicals to routine tests. The special techniques for observing effects on rate of larval growth, pupal mortality, and adult emergence, survival, and fertility are available when any special circumstance justifies their use.

Since most insecticides are soluble in Tween 20, that solvent has been routinely used in screening tests with guinea pigs. However, Radeleff & Woodard (1955) have recently found that Tween 20 injures tissues; so other solvents must be considered. Peanut oil has been used as the solvent for the chlorinated hydrocarbons injected in cattle. Most of the cattle treated with phosphorus insecticides have been given oral doses.

In routine screening the insecticides are dispersed in Tween 20 and a 1% solution or suspension is administered subcutaneously at 25 mg./kg. Even if that dose kills the guinea pig after a few hours, it is possible to make observations on its larvicidal effect, because the larvae will keep growing overnight if the animal is held at temperatures above 80° F. If a material proves lethal to the host shortly after administration, it is retested at lower doses. If it affects neither the guinea pig nor the insects at the first dose of 25 mg./kg., it may either be dropped or tried at higher doses.

When an effective subcutaneous dose is found, the same solution is given to a guinea pig orally. Some of the phosphorus insecticides were found to be effective orally and subcutaneously at about the same doses.

Treatments that show special promise in the guinea pig tests are further tested on sheep. In the oral administration of the phosphorus insecticides it has been found that chemicals effective against screw-worms in guinea pigs also work against screw-worms in sheep but that much higher doses are necessary. For example, Bayer L 13/59 killed screw-worms in guinea pigs at doses as low as 2.5 mg./kg., but in sheep approximately 20 mg./kg. was needed. Thus it is evident that not only the mode of administration but the host animal must be considered in research on a systemic insecticide for such an omnivorous species as the screw-worm.

Recent Tests Against Cattle Grubs.—We have had success in killing second- and third-instar cattle grubs with systemic treatments found effective against screw-worms in guinea pigs. Lindane, dieldrin, aldrin, Bayer L 13/59, Diazinon, and Chlorthion killed screw-worms in guinea pigs and cattle grubs encysted in the backs of cattle. However, none of these treatments were effective against first-instar cattle grubs. Chlorinated hydrocarbon and phosphorus insecticides administered in the fall when the first grubs were encysted in the back killed insects already in the second and third instars, but later in the season more larvae would migrate to the back and develop normally. Only the chlorinated hydrocarbons were tried by administration in the spring soon after cessation of heel fly activity, and those treatments always failed, as grubs appeared in normal numbers in the host animals the following fall.

The preceding paragraph is quickly read but it summarizes three years of work and illustrates one of the major difficulties in developing a systemic insecticide for cattle grubs. One has to wait too long to gather data. Since the grubs have but one generation a year, an experiment started in the spring is not completed until fall, and it is the next year before the experiment can be repeated or expanded if all the work is done at one laboratory.

In the Entomology Research Branch a little more rapid progress is possible because of the location of laboratories at Kerrville, Tex., and Corvallis, Ore. In the Kerrville area *lineatum* grubs are found in the back from August through November. Promising results obtained at Kerrville in the fall are checked at the Corvallis laboratory against *lineatum* and *bovis* in the late winter and early spring. Progress against cattle grubs is still disappointingly slow, but with the two laboratories working as a team it should be twice as fast as at only one location.

Tests Against Bloodsucking Flies.—Systemic insecticides are also considered for the control of bloodsucking flies. Knipling *et al.* (1948) killed yellow fever mosquitoes by feeding them on rabbits treated with lindane. Stable flies, *Stomoxys calcitrans* (L.), and horn flies, *Siphona irritans* (L.) were killed by feeding them on cattle injected with lindane, dieldrin, and aldrin. Eddy *et al.* (1954) found that lindane, when fed at 100 p.p.m. in the diet, made the blood of treated cattle toxic to horn flies. At Kerrville we have developed techniques for getting stable flies to engorge on guinea pigs in the laboratory, and some of the phosphorus insecticides have shown toxicity to stable flies by this method.

Lindquist *et al.* (1953) administered insecticides to white mice and then exposed them to attack by field-captured horse flies (*Tabanus* spp.), deer flies (*Chrysops discalis* Will.), and mosquitoes (*Aedes dorsalis* Meig.). Materials that showed promise in the laboratory were further checked by administration to cattle exposed to

natural populations of insects in the field. In the field they observed good kills of deer flies and mosquitoes but poor results against the horse flies.

TESTS AGAINST LICE.—Only incidental tests have been made against various species of lice. At Kerrville C. L. Smith and W. L. Barrett, Jr., found that cattle lice, *Haematopinus eurysternus* (Nitz.) and *Linognathus vituli* (L.), and the hog louse, *H. adventicius* Neum., were also killed by oral doses of 2-pivalyl-1,3-indandione, but work on this material had to be dropped because of its high mammalian toxicity.

A number of the cattle treated with insecticides in experiments against cattle grubs happened also to be infected with *vituli*. C. L. Smith observed that dieldrin, aldrin, lindane, and Diazinon given at doses effective against cattle grubs also killed the lice. He further noted that Diazinon injected in Angora goats at 10 mg./kg. killed *Bovicola caprae* (Gurlt.).

One group of guinea pigs that was used for a screw-worm experiment happened to be heavily infested with *Gliricola porcelli* (L.). Diazinon killed these lice at 10 mg./kg.

TESTS AGAINST THE LONE STAR TICK.—At Kerrville the pastures where experimental cattle are held are infested with the lone star tick, *Amblyomma americanum* (L.). In the course of experiments on cattle treated with chlorinated hydrocarbon and phosphorus insecticides these ticks appeared not to be affected. R. W. Wells found that lindane and aldrin at 50 mg./kg. and dieldrin at 25 mg./kg. had no effect, as adults crawled on the treated animals, engorged normally, and laid normal numbers of fertile eggs.

A laboratory procedure for preliminary evaluation of an insecticide against ticks has recently been devised. Unfed adult ticks are collected in the pastures adjoining the laboratory and placed in a cage with a guinea pig, to which they readily attach.

CONCLUSIONS.—A systemic insecticide that is high in insecticial efficiency but so low in mammalian toxicity that there is an adequate margin of safety for general use by farmers has not been found, but we have been partly successful in attaining this goal. Research veterinarians have followed our leads on lindane in the treatment of screw-worm infestations in horses and other animals not intended for slaughter for human food.

Because of the difficulty of injecting subcutaneously large quantities of an oil solution and as the margin of safety between an effective dose and a poisonous amount is not great, lindane as a systemic insecticide seems limited to use by professional veterinarians.

Recent success with oral doses of phosphorus insecticides makes treatment much easier than the injection method required for application of the chlorinated hydrocarbons. However, an insecticide that can be administered by the stockman either as a drench or mixed with salt or ground feed is still a goal for the future.

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Toxicological Problems in the Use of Systemic Insecticides for Livestock[1]

R. D. Radeleff and George T. Woodard, *U.S.D.A., Agr. Res. Serv., Animal Disease and Parasite Research Branch*

During the past 3 years we have treated hundreds of animals with systemically active insecticides and have observed their effects upon those animals. On the basis of this experience we would like to review a few of the problems we encountered and to point out a number of others that will appear as this work is continued.

A successful systemic insecticide for livestock will enjoy widespread use. Wide usage will make it attractive as a salable product. Those who may eventually recommend its use and those who will formulate and distribute it take upon themselves a responsibility for the protection of all who may come in contact with the product, directly or indirectly, as well as for the health of the treated animals. Both parties should be familiar with the many new considerations required in the use of such an insecticide.

Many of the toxicological problems associated with the use of a systemic insecticide in livestock are identical to those already encountered in the use of insecticides on livestock and many are peculiar to this technique. The old familiar problems have new facets to be investigated and interpreted.

Safety for the Treated Animal.—It is obvious that a systemic insecticide must be safe for the animals in which it is to be used; this is a first consideration for all livestock insecticides. To determine the toxic effects of an insecticide we may administer appropriate dosages and then study the symptoms displayed, the lesions produced or the effects of the compound upon some known and understood system or systems within the body, preferably in a readily available tissue or fluid such as fat, blood, or urine.

When an insecticide has a specific effect upon a known system as the organic phosphorus compounds have upon the enzyme cholinesterase, and that effect can be readily measured, our ability to estimate the degree of effect of a specified dosage is markedly increased. At the same time we reduce our animal losses by being able to predict the effect without depending upon objective symptoms or death for our indicator.

In the particular case of cholinesterase it has been necessary for us to establish normal values for the various farm animals. This work is by no means complete but we have enough information on cattle and sheep to proceed with some confidence. Unfortunately, while the measurement of cholinesterase inhibition can be done rather readily, the variation in cholinesterase reserves between individuals of a species is wide, some individuals will have five times as much in reserve as others. This wide variation suggests that s dosage of an inhibitor that will reduce the reserve of the highest individual by 50 per cent might completely inhibit the cholinesterase of the lowest. The wide variation in individuals will not complicate the diagnosis of poisoning by these compounds if samples of the animal's blood show residual cholinesterase to be 15 to 20 per cent of the mean for the species but will definitely preclude the assessment of a degree of damage less than active poisoning unless control samples from the animals are available.

We have noted on several occasions that the cholinesterase reserve of cattle and sheep gradually declines when the animals are transferred from pastures to feed lots. The decline in reserve may amount to as much as 40 per cent of the initial value. Whether this change is due to diet or environment or to some other cause is not known. It will be well for us to keep it in mind as a difference to be expected between range animals and those in the feed lot.

When a system such as blood cannot be studied we will need to rely on the older method of observing symptoms and lesions. Our ability to predict with this method is not as good as with the foregoing measurement of cholinesterase. The method is far more expensive because of the losses necessary and for that reason limits our studies to a relatively few animals.

No matter the method of determining the toxicity of a compound, we must always be aware of the fact that certain combinations of factors may lead to severe losses even after extensive usage has indicated complete safety. Such a combination caused the loss of nearly one-half of a band of 1,000 sheep at Brady, Texas, in 1952, that were dipped in a solution containing 0.06% gamma isomer of BHC. In that case emaciation and lactation made up the lethal combination. That loss occurred after 1,700,000 sheep had been dipped in Texas alone without losses.

It would be helpful and convenient if the toxicity data obtained in experimentation with laboratory animals could be used directly for livestock. We have learned through bitter experiemce that laboratory animals do not respond to insecticides in the same manner as livestock, and that the various species of livestock respond differently. It will therefore be necessary to study each species of farm animal as a separate case before we recommend a systemic insecticide. Our recommended compound should preferably be safe for as many species as possible and be effective at about the same dosage for each species in order to avoid the inevitable accidents that occur through failure to observe a complicated set of cautions and doses.

The closer we can come to a 10 to 1 margin of safety the more secure we will be in the safety and satisfactory performance of our material. We must always strive for a dosage that is completely safe for that most susceptible animal whose identity will not be known in advance of the treatment.

Subacute or chronic toxicity, which ordinarily infer the administration of small doses of a poison over an extended period, should not be a problem with a compound given only two or three times a year, yet must definitely be considered with many subcutaneously administered compounds. This is true because, by administering a chemical in an oil to obtain prolonged activity we are exposing the animal to a continuous small dosage. We know from autopsy findings that these oils are slowly

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absorbed and we have proved by the use of Carbon 14-labeled DDT that several months may be required for complete absorption. The significance of this low-level dosage will probably be different from compound to compound but cannot be known until investigated.

The low but continuing level of dosage following subcutaneous administration may be the beginning of a cycle with compounds that are retained in the fat or other tissues of the body. A compound having a very low rate of elimination might be administered in the spring when the animals were gaining weight and parasites were beginning to appear. Most of the absorbed compound would be stored in the fat, very little or none being lost to the organs of elimination. During late fall and early winter, as the fat depots were depleted, the compound might be released and again circulate over the body. Should the release be more rapid than the elimination, poisoning might occur. The compound could also undergo a chemical change during its transport from the subcutaneous tissues to the fat, becoming a more toxic compound. This would increase the possibility of poisoning when the subsequent release occurs. Just such a change has been observed in some animals in the conversion of heptachlor to heptachlorepoxide.

It may be that different metabolic pathways will function according to the route of administration employed or that different individuals or species may employ different pathways. According to the pathway the compound might be made either more toxic or less toxic with the effects upon the animal varying accordingly. Although such possible differences may be uncommon, they must be added to the risks involved.

RESIDUES IN ANIMAL TISSUES AND PRODUCTS.—The presence of an insecticide in the fat or in other tissues of food animals may constitute a hazard to human health. If this were not true, dieldrin, aldrin and lindane would probably already be marketed as injectable products. There was no question about their effectiveness against a large group of parasites. That it was not possible to use them can be seen from the following data. Cattle were treated a single time with dieldrin in peanut oil at 25 mg./kg. subcutaneously and biopsied 4 weeks later. Their fat contained from 44 to 155 parts per million (p.p.m.) of dieldrin at that time. In a similar manner cattle treated once with aldrin at 50 mg./kg. were found to have from 75 to 175 p.p.m. of that insecticide in their fat 4 weeks after treatment. One steer given 50 mg./kg. of lindane subcutaneously showed 54 p.p.m. in its fat 4 weeks after treatment. When four doses of these materials were given at 4-week intervals at the above dosage and the cattle biopsied 4 months after the last injection, those treated with aldrin had 188 p.p.m. in their fat, those receiving dieldrin 68 p.p.m. Lindane disappeared more rapidly, there being only 2 p.p.m. in the fat 4 months after the final dose. Such high and long-lasting residues from dieldrin and aldrin prevent their use. Lindane, because of its more rapid elimination, could be used for limited veterinary applications when the animals would not be offered for sale for several months after treatment.

A systemic insecticide might also find its way into the milk of lactating animals. With the slowly absorbed insecticides this could lead to an almost continuous contamination of the animal's milk. Compounds that are undesirable in milk from the standpoint of human consumption cannot and will not be recommended for use on dairy animals. It will be difficult to limit the use of any compound to a certain group of animals if it is really effective, therefore a useful systemic insecticide should not be excreted in milk or, if present, have any harmful effect.

Effect upon Enzyme Systems.—We are aware of the effects of some of the phosphorus insecticides on cholinesterase but we know practically nothing of their effects upon other enzymes in the tissues or in the digestive tract. These should be studied before we recommend a treatment for widespread use.

Effect upon Intestinal Organisms.—In this general field we must be alert to the possible effects of such materials upon the bacteria and othe flora of the digestive tract. While important to all animals, the gastrointestinal flora is perhaps of greatest importance to the ruminants which depend so extensively upon rumen bacteria for their digestion and well-being. This problem will be more acute when we mix the insecticide with feed, salt or mineral mixtures and less so when the parenteral routes are used. However, it is known that some chemicals are excreted with the bile into the upper intestine regardless of the manner of administration, so the effect upon intestinal flora may be observed even though the administration was subcutaneously.

PROBLEMS IN ADMINISTRATION BY INJECTION.—A mechanical hindrance to the popularity of injectable systemic insecticides is the volume of fluid that is required. To obtain a dosage of 50 mg./kg. from a 5% solution we must administer 1 milliliter of fluid for each kilogram of body weight, a total volume of 250 to 500 milliliters for an adult cow. Lindane was not toxic to cattle at 50 mg./kg. when administered as a 5% solution in peanut oil but when that concentration was doubled to reduce the volume, the same dosage did produce poisoning. If we cannot reduce the volume by increasing the concentration then we must have compounds that are effective at a much lower dosage. One may ask why the solid material cannot be used. This is possible but would also have disadvantages so long as the required dosage is high, requiring a surgical procedure for implantation. When 12 to 15 grams of insecticide are to be implanted this would be rather a difficult procedure and definitely not practical.

Subcutaneous administration presents another problem in that the material, once administered, cannot be retrieved. With dermal and oral administration there is a chance to help a poisoned animal by washing or purging but there will be none when the material has been placed under the skin and has diffused over a wide area. Treatment will, therefore, be complicated by the continuing absorption of the toxicant after poisoning is first recognized. It is probable that once poisoning has begun it will terminate fatally unless some specific antidote is at hand.

We must be cautious in the transfer of data obtained from one route of administration to another route. To illustrate this point, lindane and aldrin at 50 mg./kg. administered subcutaneously as peanut oil solutions do not cause poisoning. Either of them given orally to cattle at 25 mg./kg. is very quickly lethal. On the other hand, one of our experimental compounds produced equally severe effects whether administered orally or subcuta-

neously at identical doses.

When administered subcutaneously the chemical and its solvent or vehicle must be acceptable to the tissues, otherwise the tissues revolt and violent reactions may follow. This was illustrated when we used Tween 20 as a solvent instead of peanut oil for some of our work. In these animals a severe, diffuse cellulitis developed around each injection site immediately after administration and persisted for several days thereafter. When the injections were made in the cervical region the animals came very near to dying by strangulation. Some insecticides may produce similar reactions when given alone, eliminating them for administration in this manner.

Suitable solvents or vehicles will need to be developed according to the requirement made of the insecticide. In some instances it may be desirable to have the insecticide absorbed and eliminated rapidly in order to strike a sharp blow against a parasite without leaving a residue in the tissues. For such an objective water solutions might be employed if the chemical be soluble or a water suspension if it is not. In the latter case we will need suspending agents to assure uniformity of dosage. These agents will need to be tolerated by the tissues. In most instances a longer action would be desired and could be obtained either by absorption and storage in the tissues of the host or by administration in a vehicle that will release it over the desired period of time. For some parasites such as lice this period would need to extend only a little longer than the normal life cycle of the parasite. In such a case a different vehicle might be employed than would be used when action is required against ticks.

A serious problem must be met if it be required that the insecticide be present at the injection site for several weeks or months. Just where can we make the injection in order to be sure that any residual material is removed in routine slaughter? In the present system of using all possible parts of the carcass we will need to give this problem some real consideration. We have only to recall the unfortunate experience of the fur farmers who fed their animals upon chicken heads and necks which contained stilbesterol remaining from chemical caponization. The fur animals were rendered sterile by the stilbesterol. A partial answer might be found in the use of a totally insoluble coloring agent suspended in the injection fluid which would more or less permanently mark the injection site and require trimming before sale of the meat. The trimming required in any case may be a boomerang, requiring a greater loss by trimming of the injection site than that required by the presence of the parasite against which it was used.

PROBLEMS IN ORAL ADMINISTRATION.—If we try salt, mineral or other methods of feeding we will need a very safe material because of the irregular intake, particularly of salt and minerals.

The average consumption of salt by cattle has been estimated to be 1 ounce per day from all sources. In one of our experiments with cattle using a salt-free feed and providing measured amounts of salt for individual animals we found the consumption to vary from zero to 87 grams per week. The maximum rate would be 12.5 grams per day. For an insecticide mixed with salt at the rate of 1 part insecticide to 10 parts salt this would be a consumption of 1.25 grams of insecticide per day, or 5 mg./kg. for a 250 kilogram animal. At the lower consumption rates the daily dose would vary down to zero. In this particular study we were aware that most of the consumption took place at one or two times during the week rather than on an evenly spaced basis. This would make the dosage much higher on some days than on others.

The compatibility of the insecticide with the feed, mineral or salt with which it is incorporated will need to be explored carefully, with some attention being given to the variety of containers in which it may be fed or held. In the past, hydrolysis and other changes have tended to decrease the toxicity of insecticides but the future may bring us some whose toxicity will be increased.

While the compound will need to be palatable to the animals we must also know that the country boy who likes to eat a little feed or salt as he goes about his chores will not be poisoned should he partake of some of the treated feed or salt. Consideration must be given to the farmer's stock dog that also likes to indulge his appetite, or other species who use the salt or mineral for their nourishment.

EFFECT OF SIMULTANEOUS OPERATIONS.—The ultimate goal of all our experimentation is the development of a method that is practical and safe in the hands of the everyday farmer and rancher. What, then, will be the effect to be observed when the farmer or rancher decides to castrate, dehorn, mark, vaccinate, drench for internal parasites and administer a systemic insecticide all at the same time? This is a very real problem because the normal practice of ranchers is to do just that now, except for the systemic, and they will continue to do so. What will be the effects upon wound healing, blood coagulation, the body defenses against infection and the accumulated effects of all the operations? These will need to be determined.

OTHER VERY REAL PROBLEMS.—Our normal experimental procedure is to use animals in good condition and free of disease. What effects are we to see when we encounter nutritional anemias and mineral deficiencies? If emaciation and lactation play such an important part, can these other nutritional disturbances be ignored?

Beyond these problems are those that would require long, tedious years of study. What effects will there be on fertility and on the reproductive cycle? To what degree and with what danger will the treated dam transfer the insecticide to her offspring? Will there be harmful effects upon growth? Repeatedly suspicion has been cast upon our present insecticides along these lines; it will be cast upon systemics with more emphasis.

THE FUTURE.—We are thus surrounded on all sides by problems and considerations that tend at times to overwhelm us. We are not discouraged because we have only to think of the use of sodium fluoride in the feed of swine as an ascaricide, of phenothiazine in salt as a nemathelminthic, antibiotics in feed as growth stimulators, to realize that potent compounds can and are being regularly administered to farm animals. We are encouraged to find lead arsenate, nicotine, copper sulfate, and many other toxic compounds being routinely administered as single dose treatments. Our proposed uses of insecticides as systemics must merely be fitted to the requirements of safety and effectiveness.

Research on the Control of Forest Insects by Aircraft[1]

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The airplane was first used in the battle against forest insects by Houser (1922) and associates in Ohio 34 years ago. In that experiment for control of catalpa sphinx lead arsenate dust was released from a small hand cranked hopper secured to the outside of the fuselage of a Curtis JN6. Within a few years airplane dusting had become an established and economical procedure for application of insecticides to cotton and vegetable crops. For forest insect control, however, with the older insecticides such high application rates were required that treatment by airplane was not economical. The discovery of DDT changed this situation. Following first tests of the use of DDT sprays for control of forest insects in 1944 Craighead & Brown (1945) stated, "these experiments indicate that no development in the last 25 years has offered so much promise for the control of many forest insects, particularly defoliators." In the light of our knowledge today, this was a conservative statement.

Following World War II an intense interest in airplane spraying developed due to the phenomenal effectiveness of DDT, the large number of war surplus planes, and availability of pilots anxious to continue in aviation. The Armed Forces had been using DDT sprays and various types of spray equipment for aircraft had been hurriedly developed.

For civilian use of DDT it was necessary that the application be as economical and effective as possible with a minimum of damage to adjacent crops, fish and wildlife. There was little known about the most efficient spray equipment and operational procedures to use. Other important questions in need of answers were the effect of spray atomization, application rate, height of flight, and meteorological factors on spray distribution and insect mortality.

In order to determine, for forest insect control, the effects of some of the above factors a cooperative research project has been carried on for a number of years by the Division of Forest Insect Research, Forest Service, and the Agricultural Engineering Research Branch, Agricultural Research Service, at Beltsville, Maryland. For the past several years, research on the use of aircraft for forest insect detection has also been carried on, but the spraying work will be discussed first.

The main objective of this research has been to reduce the cost of spraying by improving the efficiency of spray equipment, spray formulations and application procedures. Most of the work has been done with Stearman and N3N planes equipped with pump and under-wing boom and nozzle systems.

These systems have been the most satisfactory of those tested and have been adapted for use on other single engine and multi-engine aircraft. The use of a pump insures a uniform rate of output and the boom permits changes in arrangement and type of nozzles to adapt the systems to various control job requirements.

Dual Spray Apparatus.—Two important factors which affect distribution of spray across a swath are degree of atomization of the material and spanwise location of the spray outlets on the plane. When two separate test flights are made, for example, to compare the effect of two different atomizations, the ever changing meteorological conditions may affect the deposit pattern to a greater extent than changes in the equipment. This problem was solved by the construction of a dual spray apparatus (Miller & Isler 1951) consisting of two complete spray systems, for test purposes. This permits the release of spray simultaneously from both systems, with the result that the effect on spray distribution of any two degrees of atomization, nozzle arrangements, spray formulations, etc., can be compared under identical meteorological conditions. By using different dyes in the spray liquid of the two systems the quantity of spray deposited from each system at any point in the spray swath can be determined by the use of a spectrophotometer.

Effect of Atomization.—A Stearman with dual spray apparatus has been used in tests to compare the effects on spray deposit of three degrees of atomization. In one series a coarse spray, 300 microns mmd (mass median diameter), was compared with a medium spray, 150 microns mmd. Then, in a second series the medium spray was compared with a fine spray, 80 microns mmd. All flights were made at an altitude of 50 ft. and upwind over open ground.

The results showed that under the conditions of the upwind tests, the coarse atomization resulted in the narrowest swath, the least uniform distribution across the swath, and in excessively high deposit peaks. The fine spray gave a slightly wider and more uniform swath than the medium spray, but this small advantage was overshadowed by the higher loss of the fine spray. It was concluded that a medium atomization produces the most efficient swath pattern for forest spraying. This conclusion has been substantiated by field control tests in which medium spray atomization was more effective than coarse or fine sprays for controlling spruce budworm.

Nozzle Arrangement.—Forest spraying has generally been done with approximately full wing span booms and nozzles. This type of equipment produces two rather high peaks of spray deposit 2 to $2\frac{1}{2}$ wing spans apart when flown upwind at 50 ft. above the ground. These heavy deposits represent inefficient spray distribution because, in general, they are considerably greater than is necessary for effective control of forest defoliators. A study has been started to determine how deposit patterns can be affected by varying the location of the nozzles along the boom. The study has two primary objectives: (1) how to lower these peaks of spray deposit; and (2) how to increase the effective swath width. A Stearman dual sprayer is being used, with a medium atomization of 150 microns mmd. for comparative test flights at 80 mph and 50 ft. altitude. A standard arrangement of nozzles is used on one boom and various experimental arrangements on the other.

From results obtained thus far, a concentration of the nozzles in the outboard one-half of the boom is not recom-

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mended because this arrangement resulted in higher peaks of excess deposit with no appreciable increase in width of swath. An interesting development in these studies has been the finding that almost identical spray deposit patterns were obtained when 24 nozzles were located either along a full wing span boom or when they were concentrated only in the inboard half of the boom. Additional tests will be made to determine whether or not the deposit peaks can be lowered without a reduction in swath width by further concentration of nozzles inboard on the boom. It appears that the pattern of airflow created by the plane plays a dominant role in the distribution of spray in the swath, particularly when flights are made 50 feet or more above the ground. The wing tip vortex action definitely assists lateral distribution of the spray, but when nozzles are concentrated near the wing tips the vortex action causes undesirable deposit peaks within the swath. Under the calm air and upwind conditions of the tests deposit peaks are probably greater and swath widths narrower than under actual field application conditions. For the latter, the terrain rather than wind direction determines direction of flight and any crosswind condition should assist lateral distribution.

We now have a Piper Cub equipped with dual spray apparatus for tests directed toward obtaining more economical spray application techniques.

NEW AIRCRAFT.—New aircraft, specifically designed for agricultural purposes, are rapidly being developed. Among these are the Ag-2, being built by Transland Co., Los Angeles; the Ag-3, developed at Texas A & M College; the Air Tractor of Central-Lamson, Yakima, Washington; the new Fletcher plane; Call Air; Taylorcraft; and others. These planes are being viewed with interest from the standpoint of replacement for the obsolete war surplus trainers now being used as well as their promise of improved and safer performance. Helicopters do a very satisfactory job of spray application, but their use on large scale control operations has been limited due to the long ferry distances involved and their higher initial and operating costs (Eaton *et al.* 1949).

OPERATING PROCEDURES.—Results of preliminary tests indicate that the flying height specified in forest spraying contracts may be increased for greater safety without reducing the effectiveness of the applications. Further field tests, however, are needed before specific recommendations can be made.

Methods for marking the swaths or guiding the plane in large scale operations would greatly increase the efficiency of the application. An interesting approach toward the solution of this problem was the formation flying (two planes working together) used the past 2 years on the Canadian spruce budworm control jobs.

EVALUATION TECHNIQUES.—Research on airplane spray deposits could be speeded up considerably by simplification of methods for measurement of quantity and atomization. The dye tracer method is being used for quantitative measurements of spray deposit when the dual sprayer is used. Recently a simple and rapid method for rough measurement of the quantity and atomization of oil spray deposits has been developed for field use. Here, special paper cards bearing an oil soluble dye coating are used to intercept the falling spray. The spray drops make circular marks on the paper and the amount of spray and its atomization are then estimated by comparing the cards with known "standards" (cards previously treated with known quantities and drop sizes). These standards are now available in printed form for field use (Davis 1954). The method is useful for checking swath pattern and atomization of commerical spray planes employed on large control projects.

A research procedure for accurately determining degree of atomization has also been developed, using the same dyed cards. This method requires only about half the time and is more accurate than the photographic method previously used.

Generally speaking, it may be said that an aerial application of insecticides is the only practical means for direct control of certain forest defoliators over large areas. Due to the low annual return per acre from such areas spraying costs must be held to a minimum. Through the efforts of various research agencies and commercial operators, the cost of controlling such insects as gypsy moth and spruce budworm has been lowered from $3.00 per acre to about $1.00 over the past 10 years. Additional savings are possible through: (1) saving of spray material by more uniform distribution and reduction of peaks of excess deposit; (2) increasing the effective width of swath by improving efficiency of spray apparatus and aircraft; and (3) development of more effective formulations. Safety may also be increased by use of some of the recently developed new aircraft and additional research may show that height of flight can be increased without reducing efficiency of the spray operation.

AIRPLANE SURVEYS.—The use of airplanes to locate and determine the intensity of insect infestations, although not new, has increased rapidly during the past few years. Research on aerial survey methods for forest insect surveys is conducted at Beltsville, Maryland, and Portland, Oregon, by the Forest Service with the cooperation of various Federal, State and private agencies.

Both visual and aerial photographic methods have been developed. Two types of visual methods in use are: (1) a line strip method (Heller *et al.* 1952) in which observers record the degree of infestation through electrically operated pens on a moving chart as the plane is flown along predetermined flight lines; and (2) sketch mapping, in which observers plot infested areas on existing aerial photos or on planimetric maps. The line strip method has been found well suited for surveying spruce budworm and larch sawfly infestations. Sketch mapping has proven highly effective for making appraisal surveys of areas infested by defoliators and some species of bark beetles.

Progress is being made in research on visual aids for aerial observers. For example, an amber-colored viewing filter has been devised which increases the observer's ability to detect discolored foliage caused by insect infestations. The filter provides a much greater contrast between green and red shades, and the effect of haze is sharply reduced. A clinometer device has also been devised for measuring, in flight, the horizontal distance from the airplane to points outboard from the flight line.

Aerial color photography, although more expensive than visual methods, has been found practical for surveys of white pine weevil damage and certain types of bark beetle infestations.

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Resistance of Lygus Bugs to DDT on Sugar Beets Grown for Seed[1]

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Lygus bugs[2] limit the production of high-quality beet seed wherever this crop is grown in the United States. Damage to beet seed by this insect was first pointed out by Hills (1941). Hills & Taylor (1950) further showed that the feeding of this insect injured very young seed but caused little damage to flower buds, open flowers, or seed approaching maturity.

Field-plot tests from 1940 to 1943 showed that either two applications of a sulfur dust impregnated with pyrethrum extract or three applications of dusting sulfur gave some control of lygus. In 1944, when DDT became available for small-plot tests, one application of a 5% DDT dust was found to be sufficient to maintain low populations throughout the season and was far superior to any other material tested.

In 1945 several tests were made with airplane applications in the Salt River Valley, Ariz. (Hills *et al.* 1946). A 5% DDT dust was applied at the rate of 30 pounds per acre to plots of 5 to 10 acres in 10 fields when the plants were in the early-bloom stage. Lygus populations were measured by means of the sweep net; 50 to 100 sweeps taken far enough into the field to get away from border effect constituted a sample. Data from these 10 fields, summarized in table 1, show that one application held populations at a very low level up to 39 days after treatment, at which time the seed were too nearly mature to be injured. The effectiveness of this one airplane application led to its adoption by the sugar-beet industry, and consequently the beet seed produced in the Salt River Valley from 1946 to 1952 was of high quality.

In 1953 germination of the seed from some of the beet fields in the Salt River Valley was unsatisfactory, and from the nature of the injury lygus bugs were suspected. Therefore, in 1954 the personnel of the Western Seed Production Corporation kept a record of lygus populations following dusting in most of the fields. These data, summarized in table 1, show that populations were satisfactorily reduced by the DDT applications but that after 10 days or 2 weeks retreatment was usually necessary. The increase was due primarily to the hatching of eggs within the fields.

Table 1.—Lygus populations in seed-beet fields before and after treatment with 30 pounds of 5% DDT dust per acre. Salt River Valley, Ariz., 1945 and 1954.

Year	Number of Fields	Mean Number of Lygus Bugs per Sweep Before Treatment	Days After Treatment		Mean Number of Lygus Bugs per Sweep After Treatment
			Range	Mean	
1945	10	4.6	1–8	5	0.3
			15–39	24	0.2
1954	19	2.5	6–9	8	0.8
			10–17[a]	13	1.3

[a] 16 fields sampled.

Table 2.—Lygus populations in field plots treated with DDT or toxaphene in Salt River Valley, Ariz., 1954.

Date	Number of Bugs per Sweep	
	5% DDT	10% Toxaphene
	Field 1	
April 20	0.6	0.4
28	2.1	2.3
May 2	Dusted	Dusted
7	0.2	0.1
13	2.0	0.2
14	Dusted	No treatment
21	0.3	0.1
	Field 2	
April 20	1.6	2.8
25	Dusted	Dusted
30	0.1	0.1
May 7	0.4	0.2
11	1.9	0.4
12	Dusted	No treatment
19	0.4	0.2

Field tests were also made in cooperation with the growers and Western Seed Production Corporation to determine the comparative effectiveness of a 5% DDT and a 10% toxaphene dust. Unreplicated plots of 10 acres each were set up in two fields, applications being made by airplane at the rate of 30 pounds per acre. Lygus samples were taken at approximately weekly intervals. These data, presented in table 2, indicate that both the dusts reduced lygus populations but that after 11 to 17 days populations in the DDT plots had reach approxi-

[1] Accepted for publication July 6, 1955.

[2] *Lygus hesperus* Knight, *lineolaris* (P. de Be.), and *elisus* Van D.

mately the same level as before treatment. To prevent excessive damage to the seed these plots were redusted with DDT. In the toxaphene plots retreatment was not necessary, and at the time of the last sampling, 19 and 24 days after treatment, the seed had matured sufficiently that it was no longer susceptible to lygus damage.

In 1954 reinfestation of the fields was due primarily to nymphs hatching from eggs deposited by adults before treatment. The same condition was obtained from 1940 to 1943 when pyrethrum dusts were used. However, tests from 1944 to 1946 indicated that the residue of DDT was sufficient to kill the small nymphs shortly after hatching. In 1954 the toxaphene residue was sufficient to kill young nymphs but not the DDT residue. This can be interpreted as evidence of development of resistance to DDT. This build-up of resistance is probably due to the exposure of this insect to DDT in cotton as well as in seed beets. Large acreages of cotton are grown in the Salt River Valley, and this crop is dusted with DDT several times each year for lygus control.

The 1954 data indicate that control of this insect in seed beets could still be obtained with two applications of DDT, but if the buildup of resistance in lygus bugs follows the same pattern that it has in other insects in only a few years it will be inadequate and some other insecticide will have to be used.

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Beet Leafhopper and Curly Top Conditions in the Southern Great Plains and Adjacent Areas[1,2]

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The beet leafhopper, *Circulifer tenellus* (Bak.), is widespread in the Western States and is an exceedingly complex problem because it transmits the virus of curly top, a disease that affects many agricultural crops. Both the insect and the virus have a wide range of hosts, consisting of at least 25 families of plants. Susceptible crops include sugar beets, garden beets, beans, eggplant, flax, mangels, melons, potatoes, peppers, spinach, squash, swiss chard, and tomatoes. Since there are 12 known strains of curly-top virus, considerable variation may occur in the symptoms of the infected plants. The only known control measures are the elimination of the weed hosts, chemical control of the insects in their weed-host breeding areas before they migrate to cultivated crops, use of virus-resistant varieties of sugar beets and beans, and cultural practices (Douglass & Cook 1954). There is no satisfactory preventive measure after the leafhoppers have invaded susceptible crops.

In 1953 sugar beets grown in southwestern Kansas were severely damaged from curly top and other causes. The average yield for the Garden City factory district was only 5.7 tons per acre, or about half a normal crop. Besides curly top the low yields were due to several adverse factors, including poor stands, root rot, a virus disease known as savoy, shortage of irrigation water, too few irrigations, hot dry weather, low soil fertility, and poor cultural practices. The growers who had fertile soil, good plant stands, and followed good cultural practices, including frequent irrigations, produced 12 tons or more per acre in 1953 as compared with 1½ tons in 1954. The low yields and high curly-top incidence created considerable interest in the leafhopper problem by the beet-sugar industry east of the Continental Divide. The industry requested that studies be made to determine the source of the beet leafhoppers that infested the area and whether or not an invasion could be expected in 1954. The results of studies made in 1954 for this purpose are reported in this paper.

KNOWN DISTRIBUTION PRIOR TO 1954.—Studies made by Romney (1939) from 1928 to 1937 showed that the spring breeding areas of the beet leafhopper in the Southwest were then confined to southern New Mexico and southwestern Texas. Douglass (1954) reported on infestations north and east of the permanent breeding areas in 1953 and showed that in that year either curly top or beet leafhoppers were found in Colorado, Illinois, Iowa, Kansas, Minnesota, New Mexico, and Wyoming (Fig. 1).

SPRING STUDIES.—Studies made from February 16 to March 8, 1954, to carry out the request of the sugar companies showed that leafhopper breeding in recent years has extended into the Edwards Plateau and the shortgrass plains area of eastern New Mexico, western Oklahoma, and northwestern Texas as far east as the 98th parallel. The migrant leafhoppers that invaded Kansas in 1953 came from these new breeding areas rather than from the old well-known breeding areas of southern New Mexico and southwestern Texas. Beet leafhoppers were found almost everywhere desirable weed-host plants occurred. Nymphs were present as far north as Sayre, Okla. The oldest ones found were in the fourth instar, which shows that they had been breeding during the winter.

When it was found that a very large area was involved, the objective was modified to determine the distribution rather than the source of overwintered leafhoppers. Incidentally the abundance was determined in several

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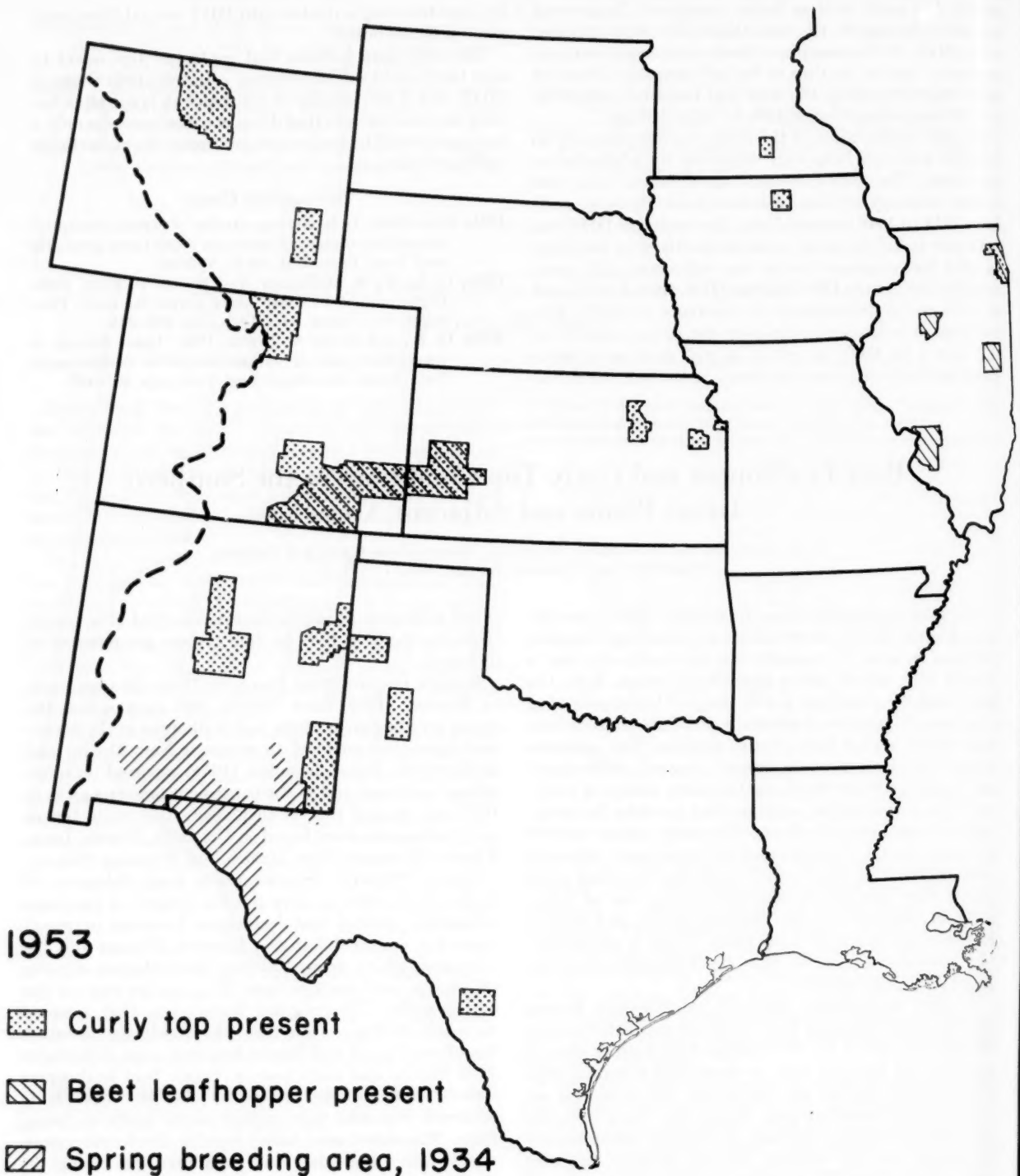

FIG. 1.—Breeding areas in New Mexico and western Texas and occurrence of beet leafhoppers or curly top in 1953 east of the Continental Divide.

places and showed average populations as high as 6.9, 1.8, and 3.5 leafhoppers per square foot of weed-host area at Pyote and Hermit, Tex., and Liberal, Kans., respectively. Figure 2 shows the distribution of the beet leafhopper as determined from this study and where the insect or curly top were reported east of the Continental Divide in 1954.

SUMMER STUDIES.—In July 1954 a study was made of the distribution of beet leafhoppers and curly top in the eastern parts of Colorado and New Mexico, northern

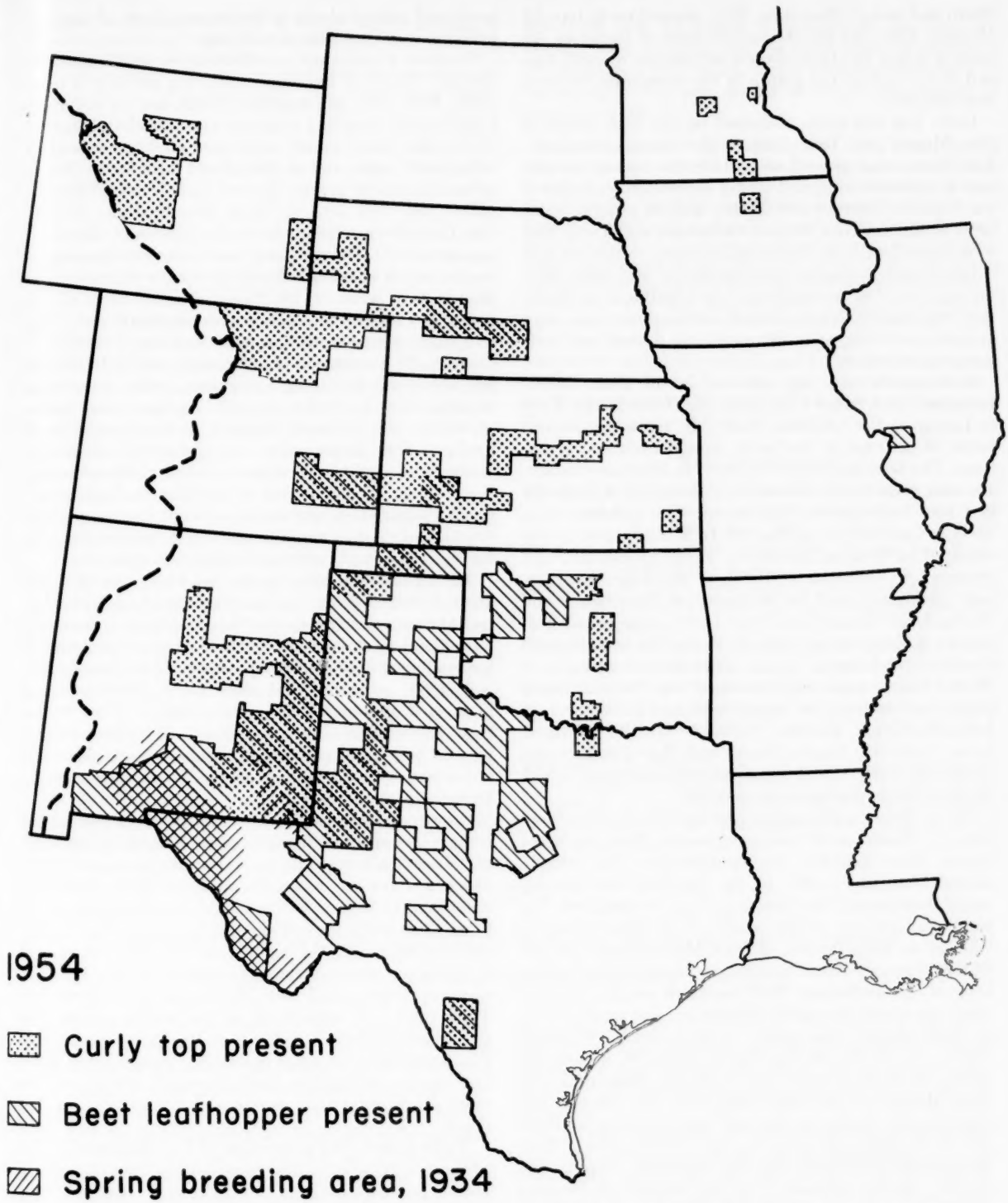

FIG. 2.—Distribution of the beet leafhopper as determined from this study and where the insect or curly top have been reported in 1954 east of the Continental Divide.

Texas, and in Oklahoma and Kansas (Fig. 2). A comparatively low but widely distributed summer population of beet leafhoppers was found. High populations were confined to a few sugar-beet and garden-beet plantings where the condition of the plants was favorable for their development. Curly top was common on garden beets, mangels, peppers, sugar beets, swiss chard, and tomatoes wherever these plants were grown. At Portales, N. Mex., almost all the commercial garden beets showed obvious curly top. Three-fourths of the sugar beets in a field at

Dawn and one at Plainview, Tex., showed curly top. At Monroe, Tex., one grower had 60 acres of tomatoes, 40 acres of which had been plowed out because of curly top, and about half of the plants in the remaining 20 acres were diseased.

Curly top was more abundant on the high plains of New Mexico and Texas than in the surrounding areas. This is the same general area where the highest population of overwintering and spring-generation leafhoppers was found in February and March 1954. In general, sugar beets in Kansas, New Mexico, Oklahoma, and Texas that were planted early on fertile soil, properly cultivated, and irrigated were in good growing condition, with little curly top and very low populations of beet leafhoppers. Fields that were planted late or did not receive proper care were in poor condition, with high curly-top disease and leafhopper populations.

In Colorado curly top occurred in the Fort Collins-Longmont and Sugar City areas, and from Rocky Ford to Lamar in the Arkansas Valley. In a few commercial fields 70 per cent of the beets showed curly-top symptoms. The beet leafhopper occurred in Madison County, Ill., and curly top in Mason City, Iowa. In Kansas the first beet leafhoppers of the season were collected from beets at Garden City on May 19. In Minnesota curly top occurred at Waseca, University Farm, Hollandale, and Stewart; in Nebraska at Hershey, Holdrege, Kearney, and Lexington; and in Wyoming at Torrington and Worland. In Texas there were heavy widespread leafhopper infestations on spinach in Zavala and Dimmit Counties, and much of the experimental spinach at Winter Haven was dying from curly top. Medium-heavy infestations on tomatoes, sugar beets, and garden beets in Lubbock, Lynn, Dawson, Howard, Martin, Midland, Ector, Andrews, Gaines, Terry, and Hale Counties and studies for curly top in these counties indicated a high incidence of the disease on these crops.

WEED HOSTS.—During the spring study beet leafhoppers were found on 37 species of weeds. Host plants not known were collected for identification. The growth ranged from the rosette to the flowering and fruiting stage; therefore, all the plants could not be identified. The plants not known by the writers were determined by William A. Dick-Peddie, of New Mexico State College. Of the 30 species determined, 22 are known to be breeding hosts of this leafhopper. Wild mustards are the principal plants on which this insect overwinters and produces one or more spring generations. Gates (1940), Waterfall (1952), and Wooten & Standley (1915) list 66, 60, and 101 species of mustards occurring in Kansas, Oklahoma, and New Mexico, respectively. Southern New Mexico and southwestern Texas are the only breeding areas in which one of the principal hosts is a perennial mustard. Both Russian-thistle (*Salsola kali* var. *tenuifolia* Tausch) and kochia (*Kochia scoparia* (L.)) are important Summer weed hosts and are widely distributed in eastern Colorado and New Mexico and in western Kansas, Oklahoma, and Texas. Russian-thistle is more important in noncultivated areas, and kochia on waste or idle lands within the cultivated areas, such as along roadsides and fence rows. There are sufficient species of weed hosts for the survival of the insect in the southern Great Plains. Nothing is known regarding the suitability of some species of mustards and related plants in this area as hosts of the beet leafhopper and the virus of curly top.

FACTORS FAVORABLE FOR EXTENSION OF WEED-HOST AREAS.—The beet leafhopper-curly top problem is man-made. It involves an introduced insect, and its important hosts in most breeding areas are also introduced species. These hosts have become established on deteriorated and overgrazed ranges and on abandoned farm lands. Native perennials, chiefly grasses, formed a stable cover when the plains were first settled. These investigations indicate that the following factors favored extension of the breeding areas: (1) Overstocking and intensive grazing of ranges, which killed or reduced the stands of grasses over thousands of acres, (2) retirement of many acres of land not suited for the production of agricultural crops and temporary abandonment of cultivated lands because of drought, (3) elimination of brush and trees by bulldozers and other soil-disturbing equipment, which encourages weed growth, (4) erosion, and (5) invasion of new species of weeds. The increased demand for the production of food and fiber during recent war years was followed by prolonged droughts and warm winters, which intensified the problem. The extension of breeding to the southern Great Plains, where new species of weed hosts are found, emphasizes the fact that the host-plant complex for both the leafhopper and curly top is constantly changing.

Throughout the loose sands, sand-hill, and shin oak areas of western Texas and eastern New Mexico, bladderpod (*Lesquerella*) and spectacle-pod (*Dithyrea*) mustards were found. Certain species of these mustards are suitable for producing high spring populations of this insect. Finnell (1939) estimated that there are 8,530,000 acres of loose sand and sand hills in the southern Great Plains. Since environments where sandy soils occur are preferred by the beet leafhopper, these could become the most important spring breeding areas of this insect in the United States.

EFFECT OF RAINFALL ON DISTRIBUTION.—Precipitation is the most important factor in controlling the distribution of the beet leafhopper. Its major breeding areas are in arid or semiarid country where the mean annual rainfall is not more than 12 inches. All the major breeding areas except those around the lower Colorado River and Rio Grande are very dry during the summer, and the heaviest rainfall is during the winter or spring (Douglass & Cook 1954). In southern Arizona and New Mexico and in southwestern Texas there is an appreciable winter rainfall and another rainy period late in the summer. In the southern Great Plains and adjacent areas practically three-fourths of the rain occurs during the growing season, which may be more detrimental than spring and winter rains to this insect.

Table 1 shows the percentage of U.S. Weather Bureau Stations in these areas that recorded less than 12 inches of annual precipitation from 1949 to 1953. In 1949 they were only 6 per cent of the stations most of which were located where the leafhopper has been known to be a pest for many years, but in 1952 they were increased to 26 per cent. In the Arkansas River drainage area of southeastern Colorado, the percentage increased from 33 in 1949 to 52 in 1952. In northeastern New Mexico it increased from 0 to 17 per cent and in northwestern Texas from 0 to 41 per cent. This drought extended to areas where spring breed-

Table 1.—Per cent of U. S. Weather Bureau stations in the southern Great Plains area reporting not more than 12 inches of annual precipitation.

STATES	NORMAL[a]	1949	1950	1951	1952	1953
Colorado[b]	16	14	52	33	61	47
Kansas	0	0	Trace	0	10	1
New Mexico	19	26	54	70	60	75
Oklahoma	0	0	0	0	5	1
Texas	2	2	6	12	18	13
Total	8	6	19	21	26	23

[a] Taken from the U. S. Dept. Agr. Yearbook, 1941.
[b] Exclusive of Colorado River drainage area.

ing of this leafhopper had not been previously recorded. Whether this apparent shift will be permanent cannot be anticipated. The occurrence of the beet leafhopper or curly top over such a wide area in 1953 and 1954 indicates a large movement of spring migrants from their southern Great Plains breeding grounds because of abnormally dry seasons and warm winters. Repetitions of such movements, however, would be unlikely in wet years and in years of normal rainfall.

SUMMARY.—In 1953 sugar beets in southwestern Kansas were severely affected by curly top, with about a 50% reduction in yield. Studies in 1954 indicated that the spring breeding areas of the beet leafhopper, *Circulifer tenellus* (Bak.), had been extended north and east of the previously known area and that curly top was widespread throughout the southern Great Plains and adjacent areas.

The factors favorable for this extension of the breeding areas were (1) overstocking and intensive grazing of ranges, which killed or reduced the stands of grasses, (2) retirement of cultivated lands because of drought, (3) destruction of brush and trees, (4) erosion, and (5) new species of weeds.

Precipitation is the most important single factor limiting the distribution of this insect. Its major breeding areas are in country where the mean annual rainfall is not more than 12 inches. Practically all the southern Great Plains normally recives more than 12 inches of precipitation a year, which should limit the importance of the insect in that area during wet seasons and years of normal rainfall.

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Cultural Practices Affecting Wireworm Injury to Potatoes[1]

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The prevention of wireworm injury to potatoes has always been an important but difficult problem. Injuries to the tubers by even very low populations of these pests may seriously reduce the market value of the crop. The amount of injury is related to the number of wireworms in the soil, but other factors may affect their depredations (Gibson 1939). The value of planting late in the season in New York to reduce injury to the crop was reported by Rawlins (1939). Jones & Shirck (1942) found that under irrigated conditions in Washington late-June plantings were effective in minimizing wireworm injury to potato tubers. The effects of different quantities of irrigation water and different planting times on wireworm injury were studied near Walla Walla, Wash., from 1934 to 1937.

METHODS.—One-twentieth-acre plots of silt loam soil were set up in a field that had been planted to truck or forage crops for several years. The Pacific Coast wireworm, *Limonius canus* Lec., comprised about 90 per cent of the wireworm population, and the sugar-beet wireworm, *L. californicus* Mann., was of secondary importance.

Population estimates of both larvae and adults were made each spring before the potatoes were planted and again in the fall after harvest. Twenty-five soil samples in each plot were dug and sifted and the wireworms counted. Each sample was 1 foot square and 18 inches deep, and the soil was removed in 3-inch layers to determine the vertical distribution of the wireworms.

Netted Gem potatoes were planted in all the plots. At regular intervals during the season, 10 plants were dug

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Table 1.—Spring and fall populations of wireworms per square foot in experimental plots of potatoes, 1935–7.

Year	Cultural Treatment	March		October–November	
		Larvae	Adults	Larvae	Adults
1935	Minimum irrigation	2.5	0.1	1.8	1.3
	Normal irrigation	2.2	.1	1.9	0.4
1936	Planted April 7	2.0	.6	4.2	.6
	Planted June 23	2.0	.9	4.4	.6
1937	Planted May 4	2.1	.4	4.3	.4
	Planted May 20	1.9	.3	4.9	.4
	Planted June 5	2.1	.2	4.7	1.5
	Planted June 20	3.7	.2	4.7	0.4

in each plot and the tubers examined for wireworm holes. Tubers with one or more holes were considered injured, and no attempt was made to classify the degree of injury. Soil samples were also taken from the spot where each plant was removed to determine the number and depth of the wireworms around the plants at that time. The successive samplings showed the seasonal trend of wireworm abundance, distribution, and injury to the tubers.

The amount of irrigation water was determined by means of a measuring flume with a 3-inch throat placed at the head of each plot, as described by Parshall (1932). The runoff was measured with a 90-degree triangular-notch weir placed at the lower end of each plot. The amount of water actually absorbed by each plot was then readily calculated.

1934 EXPERIMENTS.—Different amounts of irrigation water were applied to eight nonreplicated plots that were planted to potatoes on April 21. The total amounts for the season ranged from 11.7 to 41.6 acre-inches per plot.

Wireworm populations in these plots increased between spring and fall. The number of larvae increased from 2.7 to 5.5 and of adults from 0.2 to 0.8 per square foot. The larval increase was due to the new brood that hatched in the latter part of May from eggs in the soil. The adult increase was the current season's production of adults, which emerge in the soil annually during July and August.

Potato samples were dug at weekly intervals from July 26 until September 25, when the total remaining crop was harvested. The sample from each plot on each sampling date weighed from 100 to 120 pounds. Although more wireworm injury occurred in the heavily irrigated plots, the differences bore a more direct relation to the population than to the amount of water applied. Plots that had about equal populations in the spring showed greater injury to the tubers where a greater population increase was shown during the season.

The injury to the tubers ranged from 51.1 to 59.3 per cent, and was 53.7 per cent on September 25, when the crop was harvested. There was no increase in injury between July 26 and September 25 in any of the plots.

1935 EXPERIMENTS.—Experiments were carried out to compare injury in plots that were planted early and late. Eight plots were used and, to avoid any differences due to uneven distribution of wireworms, alternate rows in each plot were planted on March 19 and June 23 and were designated as early and late crops, respectively. Tubers set on the early crop in June, but not on the late crop until August 1 or later. A comparison was also made between four of the plots that received a minimum of irrigation water and four that received a normal amount. There were four replicates of each of the four cultural conditions—early and late plantings with minimum and normal irrigation. In the plots that received minimum irrigation, the early-planted rows received no water, and the late-planted rows received 5.0 inches on August 30. The plots that were irrigated normally received water every 12 days from May 14 until final harvest, a total of 49.6 acre-inches being applied during the season.

Adult wireworm populations increased from spring to fall, but the larval populations decreased (Table 1). Either very few eggs were laid in the spring or the mortality of eggs and newly hatched larvae was unusually high. Usually there is an increase in larval population following one season of potatoes.

In the early crop, tuber sampling to determine wireworm injury was begun on June 24 and repeated at 2- to 3-week intervals until August 22, when final harvest was made. Sampling in the late crop was begun on September 12 and repeated until final harvest on October 25. The results are shown in table 2.

In the early crop the total injury increased consistently until the end of July in all the plots. There was a greater accumulation of wireworms about the plants, and the total injury was greater in the plots that were normally irrigated. The tuber injury per worm was slightly but consistently higher in the nonirrigated plots. No doubt the larvae preferred the moist tubers to the drier surrounding soil.

In the late crop there was no increase in injury from September 12 to October 25. The number of wireworms per plant, the total injury, and the injury per worm were very similar in the plots receiving a minimum of irrigation water and those normally irrigated. Even if the minimum irrigation had shown promise in reducing wireworm damage, it would not have been practical, for the small, misshapen tubers were of no market value.

Figures from the final harvests showed that significantly greater injury was done to the tubers in the early plantings than in the late plantings, with both minimum and normal irrigation.

1936 EXPERIMENTS.—In further experiments with early and late crops, 12 plots of potatoes were grown, half of

Table 2.—Seasonal changes in wireworm populations and injury to potatoes grown for early and late crops with minimum and normal irrigation, 1935.

Sampling Date	Number of Wireworms per Plant		Per Cent of Tubers Injured			
			Total		Average per Wireworm per Square Foot	
	Minimum	Normal	Minimum	Normal	Minimum	Normal
Early crop:						
June 24	3.8	5.6	28.5	34.2	7.5	6.1
July 11	2.7	5.2	36.0	40.8	13.3	7.8
31	2.9	4.1	50.6	61.5	17.5	15.0
Aug. 12	3.0	4.2	45.5	58.5	15.2	13.9
22	—	—	40.5	56.1	—	—
Late crop:						
Sept. 12	2.3	3.0	25.7	34.4	11.2	11.5
Oct. 2	4.0	3.5	31.6	30.1	7.9	8.6
25	—	—	19.9	27.5	—	—

Table 3.—Seasonal changes in wireworm populations and injury to potatoes grown for early and late crops, 1936.

Sampling Date	Number of Wireworms per Plant	Per Cent of Tubers Injured	
		Total	Average per Wireworm per Square Foot
Early crop:			
June 8	3.0	21.8	7.3
29	4.1	53.7	13.1
July 21	3.4	60.5	17.8
Aug. 4	2.7	63.0	23.3
15	—	56.7	—
Late crop:			
Aug. 24	3.8	42.8	11.3
Sept. 5	3.9	51.1	13.1
25	5.0	41.3	8.3
Oct. 8	6.6	49.9	7.6
Nov. 5	—	43.5	—

them planted on April 7 and the other half on June 23. Wireworm-population records before the crop was planted and after harvest were obtained by soil siftings (Table 1). Populations in both crops increased from spring to fall, but there was no appreciable change in the adult populations. This differed from 1935, when there was an increase in adults from spring to fall.

The plots were irrigated at 12-day intervals from May 14 to September 11. Tuber sampling to determine wireworm injury was begun about 2 months after planting and repeated every 2-3 weeks until final harvest. The results of these tests are shown in table 3.

In the early crop the percentage of tubers injured reached a maximum about the first of August, as in 1934 and 1935. However, the injury per worm increased steadily during the entire season. Possibly some of the larvae fed on the tubers and then left the vicinity of the plants and were not counted when the population per plant was determined. The result would be a relatively high figure for injury per worm.

In the late crop there was no appreciable or consistent increase in total injury or in injury per worm. The injury per worm was actually lower the latter part of the season than at the corresponding period in the early crop, although the population per plant was generally greater. This indicated reduced feeding prior to the first sampling. As in 1935, significantly more injury was done to the early than to the late crop.

1937 EXPERIMENTS.—Work was done to determine more precisely the effect of various planting dates on wireworm injury. Four nonreplicated plots were planted—on May 4, May 20, June 5, and June 20. The plots were irrigated at 6-day intervals during the season and harvested in the same order in which they were planted—on September 9, September 29, October 21, and November 4. The wireworm populations were determined in March before planting and again in November after all the potatoes had been harvested.

As shown in table 1, the first three plantings had nearly identical larval populations in March, but the plot planted last was more heavily infested. The adult increase from spring to fall was slight, but the larval population more than doubled in the first three plantings.

Table 4.—Wireworm injury to potato tubers found in samples collected at intervals from July 7 to November 4, 1937.

Planting Date	Sampling Date	Number of Wireworms per Plant	Per Cent of Tubers Injured	
			Total	Average per Wireworm per Square Foot
May 4	July 7	2.3	44.9	19.5
	20	2.3	72.6	31.6
	Aug. 3	3.3	52.3	15.9
	16	3.2	61.6	19.3
	Sept. 1	7.4	48.2	6.5
	9	—	76.4	—
May 20	July 20	2.7	20.6	7.6
	Aug. 3	3.5	50.8	14.6
	16	2.3	34.0	14.8
	Sept. 1	5.9	65.6	11.1
	22	7.2	38.6	5.4
	29	—	45.9	—
June 5	Aug. 16	5.0	42.4	8.5
	Sept. 1	6.6	34.0	5.2
	9	7.2	54.8	7.6
	22	10.2	61.7	6.1
	Oct. 7	12.0	47.4	4.0
	21	—	32.9	—
June 20	Sept. 1	5.8	36.1	6.2
	9	6.6	15.9	2.4
	22	5.8	8.4	1.5
	Oct. 7	9.8	12.1	1.2
	21	11.4	26.6	2.3
	Nov. 4	—	24.6	—

The results of tuber sampling between July 7 and November 4 are shown in table 4. There was no consistent correlation at any given time between the number of wireworms per plant and the injury to the tubers. There was doubtless considerable random movement of the wireworms, and the population per plant was not constant. Although there was an accumulation of larvae at the plants as the season progressed, injury to the tubers was less in the later plantings. Examinations made at final harvest showed a decrease in total injury as the planting date was advanced.

DEPTH DISTRIBUTION OF WIREWORMS.—A study was made of the depth distribution of the larvae during the 1935, 1936, and 1937 seasons, when soil siftings were made to determine the wireworm population per plant. The results are shown in table 5.

In July and August many of the larvae moved below the top 6 inches of soil, where most of the tubers were formed. Plantings made on or after June 20 did not set tubers until after August 1, and usually by this date there was material reduction in numbers in the top 6 inches, with consequent less injury to the later plantings. However, this downward migration did not account entirely for the reduced injury to potatoes planted for a late crop. In 1937 there were 1.3 wireworms per square foot in the top 6 inches of soil on August 3, 2.1 on August 15, 3.1 on September 1, and 1.6 as late as November 4. Such numbers of feeding larvae would do much more than the 24.6 per cent of total injury found at final harvest of the June 20 planting. There was a general reduction in feeding of the larvae during September and

Table 5.—Depth distribution of wireworms in experimental potato plots, 1935–7.

Date Sampled	0–3 Inches		3–6 Inches		6–9 Inches		9–12 Inches		12–18 Inches	
	Larvae per Square Foot	Per Cent of Total	Larvae per Square Foot	Per Cent of Total	Larvae per Square Foot	Per Cent of Total	Larvae per Square Foot	Per Cent of Total	Larvae per Square Foot	Per Cent of Total
1935 (80-square-foot samples)										
June 24	1.5	31.5	2.3	49.1	0.8	16.8	0.1	2.6	0	0
July 10	1.0	25.0	2.1	52.5	.7	18.4	.2	4.1	0	0
26	0.7	19.1	1.8	50.3	.8	23.4	.3	7.2	0	0
Aug. 15	.4	9.7	1.7	47.6	1.2	33.3	.3	9.4	0	0
Sept. 15	.1	4.1	0.9	26.8	1.5	45.4	.6	17.8	0.2	5.9
30	.1	1.8	1.3	27.3	1.9	38.3	1.3	25.7	.3	6.9
1936 (120-square-foot samples)										
June 9	0.4	19.4	0.8	45.6	0.5	26.7	0.1	7.4	0.01	0.9
July 9	.6	25.2	1.3	53.0	.5	18.9	.1	2.6	.01	.3
21	.2	10.8	0.9	41.0	.8	36.2	.2	10.5	.03	1.5
Aug. 10	.1	6.9	.6	32.1	.9	51.8	.2	8.3	.01	0.9
24	.1	5.4	.4	19.2	1.0	44.8	.6	25.3	.1	5.3
Sept. 8	.1	3.4	.4	20.2	1.1	48.3	.5	24.3	.1	3.8
24	.1	2.7	.6	22.8	1.4	48.7	.7	23.7	.1	2.1
Oct. 10	.1	1.5	.6	14.8	1.6	42.8	1.3	34.6	.2	6.3
1937 (40-square-foot samples)										
July 7	0.2	34.8	0.3	47.8	0.1	13.0	0.03	4.4	0	0
20	.3	26.0	.5	36.0	.3	26.0	.2	12.0	0	0
Aug. 3	.4	21.6	.9	45.9	.5	28.4	.1	4.1	0	0
15	.6	18.2	1.5	43.1	1.1	31.4	.3	7.3	0	0
Sept. 1	.8	10.3	2.3	31.8	2.7	36.7	1.3	17.1	0.3	4.1
9	.3	7.4	1.1	26.5	1.2	30.3	1.15	28.4	.3	7.4
22	.2	2.6	1.4	20.2	2.6	39.0	1.9	28.5	.7	9.7
30	.2	4.4	0.9	26.7	1.2	34.1	0.9	26.7	.3	8.1
Oct. 7	.3	3.0	1.7	22.5	3.0	39.1	1.8	23.5	.9	11.9
14	.5	10.7	1.0	22.5	1.8	39.3	0.9	19.7	.4	7.8
21	.3	5.8	1.1	20.1	2.0	35.3	1.2	21.9	1.0	16.9
29	.1	2.1	0.6	12.6	1.7	35.8	1.5	30.6	0.9	18.9
Nov. 4	.5	8.6	1.1	18.9	1.9	34.2	1.5	26.1	.7	12.2

early October, regardless of their depth in the soil.

SUMMARY.—Field-plot experiments were conducted at Walla Walla, Wash., from 1934 to 1937 to determine the effects of different planting dates and different quantities of irrigation water on injury to the potato crop by wireworms, principally, the Pacific Coast wireworm, *Limonius canus* Lec.

Generally more wireworms were found in soil samples as the season advanced and as the wireworms of the new generation increased in size.

Reductions in irrigation water tended to reduce wireworm injury but also reduced the value of the crop and did not give practical control.

Injury was progressively less as the planting date was delayed, particularly to June 20 or later. This reduction in injury was probably due to a downward movement of the larvae from the top 6 inches of soil to avoid high temperatures.

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Field Evaluation of Malathion for Control of California Red Scale on Citrus[1]

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The introduction of organic phosphorus compounds for use against scale pests on citrus in California was effected with parathion (Carman & Ewart 1950), a compound highly toxic to warm-blooded animals. In the ensuing search for safer compounds of this type with minimal or no diminution of insecticidal value, 0,0-dimethyl-S-(1,2-dicarboxyethyl) dithiophosphate (malathion) has been found to be of particular interest. This material was first reported by Johnson *et al.* (1952) to be significantly less toxic to mammalian species than parathion and many of the other economic poisons then in use, but to have appreciable toxicity against many insect and mite species.

In the initial laboratory and field trials undertaken for the control of California red scale, *Aonidiella aurantii* (Mask.), on citrus in California, malathion was found to be sufficiently effective to warrant more definitive studies. It is the purpose of this paper to present some of the data accumulated in these later studies, which will be helpful in comparing the effectiveness of malathion with that of established California red scale treatments utilizing petroleum oils and/or parathion. Additional data are presented to demonstrate the importance of various factors in the practical use of malathion, and to indicate which factors are principally limiting to such usage.

Materials and Methods.—Technical grade malathion was used in preparing the malathion formulations included in these studies, and all formulated materials were prepared by the American Cyanamid Company. Parathion materials used in comparison trials were also prepared and supplied by this company. Petroleum oils were obtained through normal supply channels and in all cases conformed to the specifications of the California Department of Agriculture classification (Erb 1932, Marshall 1932).

In most of the studies herein reported conventional citrus spray equipment utilizing a high-pressure reciprocating-type pump operating at a pressure of 550 p.s.i. was used in applying the finished sprays. The apertures of the discs used in the single-nozzle spray guns were 8/64 inch in diameter. The sprays were applied manually to both the inside and the outside of the tree from the ground, and to the outside of the tree from the catwalk of a hydraulic spray tower positioned above the tree. In some of the more recent studies an oscillating boom sprayer unit was used in making full-coverage applications of the experimental treatments. The unit used was equipped with a high-speed centrifugal pump capable of supplying spray in amounts considerably in excess of 100 gallons per minute at pressures comparable to those employed in the manual spray operations. The repetitive cyclic movement of 10 oscillating spray guns spaced 20 inches apart on a vertical boom column, coupled with the slow forward progression of the equipment in the tree row and with the spray pressure, projected the spray against the tree in a pattern characterized by both vertical and horizontal overlappings, and in a manner which effected thorough wetting of all aerial portions of the tree. Rigid control of all operating conditions was maintained to achieve the degree of coverage considered necessary for the control of California red scale.

In additional studies spray-blower equipment of the Master Fan type was used to apply malathion sprays at a relatively low volume per tree. In the unit used in these field tests air supplied by a centrifugal-type blower 30 inches in diameter was discharged on both sides of the tree row through narrow vertical "fishtail" outlets 9 feet in length. The air was supplied in large volume but with velocities of approximately 60 miles per hour only. Spray under pressures of 400 to 500 p.s.i. was discharged into the air stream through nozzles located at the extremity of the air outlet. A relatively uniform distribution of spray droplets to all parts of the tree was achieved, but the low volumes used did not normally permit complete wetting of the tree surfaces (French 1938).

The groves used in these studies were located in Los Angeles, Riverside, and San Bernardino counties and were selected with regard both to the uniformity of the trees in size, shape, and general vigor and to the generally high density of the California red scale populations.

Although some tests, particularly those involving the use of mechanical spray equipment, were made without the benefit of replication of plots, most of the tests were replicated in patterns ranging from 6 to 8 single-tree subplots to 3 replicates of multiple-tree subplots.

Pretreatment and posttreatment populations of California red scale were assayed in several different ways. In the majority of cases the data were obtained by determining the percentage of specific tree units that were infested with scale. Forty fruits, leaves, or terminal green-wood twigs were selected at random from a zone 3 to 6 feet from the ground in the peripheral area of each count tree and were critically examined for the presence of live adult female scale. The flush-growth cycles of the green-wood terminals were not included in the examined areas, which were confined to the two adjacent growth cycles, regardless of their actual length. Results of such assays are expressed in terms of the percentage of tree units infested with live adult female scale. In limited instances, the actual numbers of live adult female scale found on tree units selected in the manner described above were recorded and the results expressed in terms of the number of such scales per tree unit.

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Table 1.—Effect of annual applications of various dosages of malathion for control of California red scale on navel orange trees at Riverside, California, as indicated by percentage of fruits and leaves infested with live adult female scale and by number of scales on terminal green wood before and after spray applications of October 26, 1951, September 17, 1952 and 1953, and August 17, 1954.

Material	Amount per 100 Gallons	Count Unit	Fruit and Leaf = Per Cent Units Infested Green Wood = No. of Scales per Terminal Unit							C.R.I.[a]
			10/26/51	8/29/52	9/8/53	11/25/53	3/31/54	7/27/54	12/16/54	
Malathion 25% WP	0.5 lb.	Fruit	35.3	41.6	14.7	25.2	7.6	2.3	16.1	564
		Leaf	—	20.3	6.6	0.6	—	0.9	0.9	—
		Green wood	0.2	0.4	0.1	0.0+	—	0.0+	0.0+	—
	1.0 lb.	Fruit	38.8	48.9	15.6	12.5	1.9	1.2	4.0	402
		Leaf	—	19.1	4.1	0.9	—	1.2	0.0	—
		Green wood	0.3	0.3	0.1	0.0+	—	0.0+	0.0	—
	2.0 lb.	Fruit	42.5	28.4	8.1	5.0	0.6	0.0	0.0	184
		Leaf	—	9.1	2.5	0.0	—	0.3	0.0	—
		Green wood	0.4	0.2	0.0+	0.0+	—	0.0+	0.0	—
	4.0 lb.	Fruit	50.9	32.9	3.1	1.6	0.6	0.4	0.4	142
		Leaf	—	14.1	0.9	0.0	—	0.0	0.0	—
		Green wood	0.5	0.1	0.1	0.0	—	0.0+	0.0	—
Petroleum oil, light-medium grade, emulsive type	1.75 gal.	Fruit	39.1	15.4	1.9	0.0	0.6	0.0	3.2	100
		Leaf	—	3.1	0.3	0.0	—	0.6	0.0	—
		Green wood	0.3	0.2	0.0+	0.0	—	0.0+	0.0+	—

[a] Corrected relative infestation (Ebeling 1947); average of all posttreatment counts; 100 = results with light-medium-grade oil at a concentration of 1.75 gallon per 100 gallons.

Eight to twelve trees were used for scale counts in evaluating the effectiveness of each treatment. Pretreatment counts were made in order to determine the relative population densities in individual plot areas. Posttreatment counts were made at intervals after the application of treatments, with the ultimate objective of securing a terminal count approximately 1 year after the initial treatment, thereby permitting an evaluation of treatment over the course of an entire climatological and significant ecological cycle, and also over a period of practical significance to growers.

RESULTS.—Initial field tests indicated that malathion

Table 2.—Influence of dosage and of time, number, and sequence of applications of malathion for control of California red scale on navel orange trees at Riverside, California, as indicated by percentage of fruit infested with live adult female scale before and after indicated spray applications.

Amount per 100 Gallons	Application			Per Cent Fruit Infested					C.R.I.[a]
	3/16/53	5/15/53	7/15/53	3/16/53	11/24/53	3/31/54	7/13/54	10/6/54	
Pounds				Malathion 25 Per Cent Wettable Powder					
1.0	x			66.5	31.0	21.3	1.4	23.3	105
1.0		x		50.0	5.5	3.7	0.6	23.3	60
1.0			x	42.1	6.7	1.0	0.0	17.7	55
1.0	x	x		50.6	3.0	2.3	0.0	11.8	31
1.0	x		x	45.7	1.2	0.0	0.0	12.4	27
1.0		x	x	60.4	3.2	0.0	0.0	6.4	14
1.5	x			54.9	15.3	17.7	3.1	17.7	89
1.5		x		64.0	4.3	0.6	0.0	17.1	31
1.5			x	48.4	6.4	8.0	1.7	7.3	44
1.5	x	x		38.4	1.6	0.0	0.0	5.0	16
1.5	x		x	39.9	0.6	0.0	0.7	6.6	18
1.5		x	x	26.8	0.0	0.0	2.5	9.8	42
2.0	x			42.1	5.4	4.3	1.9	22.6	74
2.0		x		60.4	2.4	3.5	1.2	17.1	36
2.0			x	53.6	5.7	4.1	0.0	12.2	37
2.0	x	x		63.6	1.8	0.0	0.0	6.1	11
2.0	x		x	49.6	3.2	0.8	1.6	11.4	31
2.0		x	x	49.4	1.2	0.0	0.0	7.3	16
Pounds				Parathion 25 Per Cent Wettable Powder					
1.5	x			34.1	0.6	2.4	0.0	13.0	42
1.5		x		24.6	1.2	0.0	0.0	7.8	33
1.5			x	40.2	0.7	0.0	0.0	13.8	33
Gallons				Petroleum Oil, Light-medium Grade (Emulsion)					
2.0			x	40.9	4.3	2.7	0.0	38.2	100

[a] Corrected relative infestation (Ebeling 1947); average of all posttreatment counts; 100 = results with light-medium-grade oil at a concentration of 2 gallons per 100 gallons.

Table 3.—Relative effectiveness of malathion used alone and in combination with parathion and with horticultural grade kerosene for control of California red scale on Valencia orange trees at Bryn Mawr, California, as indicated by percentage of fruits infested with live adult female scale before and after spray applications of May 21, 1952.

Material/100 Gallons				Per Cent Fruit Infested			
Malathion 25% WP Pounds	Parathion 25% WP Pounds	Kerosene, Gallons	Blood Albumin Spreader, Ounces	3/27/52	10/23/52	5/19/53	C.R.I.[a] 5/19/53
1.0	—	—	—	87.7	7.4	5.7	216
2.0	—	—	—	89.1	5.7	5.7	213
3.0	—	—	—	88.6	2.6	2.6	97
0.5	0.5	—	—	84.0	4.0	6.9	273
0.5	0.75	—	—	95.4	4.3	6.3	219
0.5	1.0	—	—	93.1	5.7	6.9	246
0.75	0.5	—	—	92.6	6.3	7.1	255
0.75	0.75	—	—	82.3	4.0	4.9	198
0.75	1.0	—	—	87.7	4.3	3.1	117
1.0	0.5	—	—	89.4	6.9	5.7	212
1.0	0.75	—	—	91.1	3.4	2.0	73
1.0	1.0	—	—	91.4	2.9	2.7	98
—	1.5	—	—	89.7	3.7	2.7	100
1.0	—	1.0	4.0	94.3	8.3	20.9	736
0.5	—	2.0	4.0	96.9	9.7	16.3	559
1.0	—	2.0	0.0	94.6	10.3	15.4	541
1.0	—	2.0	4.0	94.6	6.3	12.6	442
1.0	—	2.0	8.0	94.0	7.1	14.0	495
1.5	—	2.0	4.0	93.1	5.1	11.7	417
1.0	—	3.0	4.0	88.3	6.6	7.1	267
—	1.0	2.0	4.0	93.1	6.6	9.4	335

[a] Corrected relative infestation (Ebeling 1947); 100 = results with parathion 25 per cent wettable powder at a concentration of 1.5 pounds per 100 gallons.

might be an effective treatment for the control of California red scale. One of the first expanded field tests was established in a navel orange orchard on University property in Riverside for the purpose of comparing results of a progression of malathion dosages with those of a conventional petroleum oil treatment, the treatments being repeated on an annual basis. Results of this test up to the present time are shown in table 1. Scale population estimates have been made on fruit, green wood, and leaves for the purpose of demonstrating long-term population trends rather than differential kills on these tree units.

Since it was observed in the initial studies that malathion was somewhat less effective than parathion, the feasibility of using two or more applications, with relatively short intervals between applications, was considered. In a test designed to evaluate such schedules it was also possible to involve several dosages of malathion and to arrange for a comparison of applications made (1) prior to the bloom period, (2) immediately after the bloom period, and (3) at a later date such that the intervals between treatments would be comparable. This period of the year was selected for evaluation of such multiple-treatment schedules because (1) parathion has been found to be most effective when applied during the immediate postbloom period and (2) the use of multiple treatments during this period could be more easily justified because of the possibilities of simultaneously controlling certain other pests or of including nutritional spray materials. Results of malathion treatments based upon these considerations are compared with those of parathion and of petroleum oil treatments in table 2. The posttreatment count made on March 31, 1954, is of particular significance because this count was completed just prior to the picking of the crop that was set after or during the time of the applications.

In further consideration of the possible uses of malathion for armored scale control on citrus the efficiency of combinations of this material with other effective materials was evaluated. Various combinations with parathion were tested, since previous laboratory studies had indicated their effectiveness against California red scale (Pappas & Carman 1952). Results recently published by Elmer & Ewart (1954) have also shown such mixtures to be highly effective for the control of the soft scale, *Coccus hesperidum* L., whereas parathion used alone was conducive to posttreatment increases of such populations. The reduction of parathion dosages effected by the inclusion of malathion provided a theoretical factor of safety. Typical results are presented in table 3.

Combinations of malathion with various grades of petroleum oil were also tested to determine the efficiency of such treatments in comparison with the independent use of each. The results presented in table 4 are based on a 2-year study and involve comparisons of the grades, types, and amounts of petroleum oil and the relative amounts of malathion used alone or as additives to the petroleum oils. Essentially duplicate grove tests on lemons

Table 4.—Effect of annual applications of malathion used alone or in various combinations with petroleum oils, and of reference treatments for control of California red scale on Valencia orange trees at San Fernando, California, as indicated by percentage of fruits infested with live adult female scale before and after spray applications of August 21, 1952, and September 9, 1953.

Material/100 Gallons				Per Cent Fruit Infested						
Petroleum Oil		Malathion 25% WP, Pounds	Parathion 25% WP, Pounds							
Classification and Type	Gallons			8/20/52	2/19/53	7/14/53	2/2/54	5/25/54	8/6/54	C.R.I.[a]
Light-medium, emulsive	1.75	—	—	84.2	33.3	56.1	30.8	38.9	88.1	100
Light, straight	1.75	—	0.5	91.1	5.5	14.4	0.0	0.6	3.9	9
Light, straight	1.75	0.5	—	80.6	17.2	34.7	2.2	2.8	11.7	29
Light, straight	1.75	1.0	—	64.7	13.9	19.7	0.3	0.9	3.1	20
Light, straight	1.75	1.5	—	79.4	4.4	15.0	0.0	0.4	0.3	9
Light, straight	1.25	1.0	—	71.7	19.4	31.1	1.7	0.6	9.4	30
Light, straight	0.75	1.0	—	71.4	19.2	30.9	4.7	0.9	7.5	30
Kerosene, straight	2.0	1.5	—	93.3	3.3	43.9	4.5	3.9	16.4	26
Light, straight	1.75	0.5	—	80.6	17.2	34.7	2.2	2.8	11.7	29
Light-medium, straight	1.75	0.5	—	85.6	10.5	29.7	0.6	2.8	10.3	21
Light-medium, emulsive	1.75	0.5	—	88.0	8.6	25.8	2.2	2.2	11.9	20
Light-medium, emulsion	2.0	0.5	—	77.5	14.4	25.6	0.6	1.1	4.9	20
Medium, straight	1.75	0.5	—	95.3	17.2	35.3	2.5	5.6	16.1	27
—	—	1.5	—	94.4	30.3	53.1	5.8	5.3	21.4	42
—	—	2.0	—	75.8	15.5	26.7	1.4	1.9	11.4	26
—	—	2.5	—	96.1	27.5	46.9	5.6	3.6	16.4	35
—	—	3.0	—	69.2	12.5	23.6	1.7	3.3	12.5	26
—	—	4.0	—	60.3	10.5	12.2	1.1	0.4	2.1	15
—	—	—	2.0	89.2	27.5	29.2	3.6	3.9	11.1	29

[a] Corrected relative infestation (Ebeling 1947); average of all posttreatment counts; 100 = results with light-medium-grade oil at a concentration of 1.75 gallons per 100 gallons.

based upon the results of the earlier field trials with malathion and petroleum oil combinations are shown in table 5. The evaluation of kerosene as an adjunct to malathion in sprays for the control of California red scale was additionally undertaken, since this grade of petroleum oil has been shown by various workers to be less likely to cause adverse tree reactions of the sort frequently attributable to the use of the heavier grades of petroleum oil. It was also conjectured that kerosene might enhance the effectiveness of malathion sufficiently to justify limited dosage reductions. Results of tests with kerosene-malathion sprays are included in table 3.

Because of the tendency for malathion to contribute significantly to California red scale control but to be somewhat less effective than parathion, it was of value to test the possibilities of incorporating malathion into

Table 5.—Comparative effectiveness of semiannual applications of malathion used alone or in combination with petroleum oil for control of California red scale on Eureka lemon trees at two locations, as indicated by percentage of fruit infested with live adult female scale before and after spray applications of October 8–9, 1953, and April 29, 1954.

Material/100 Gallons				Per Cent Fruits Infested			C.R.I.[a]	
Petroleum Oil		Malathion 25% WP, Pounds	Parathion 25% WP, Pounds					
Classification and Type	Gallons			10/7/53	2/24/54	9/22/54	2/24/54	9/22/54
Grove A, Granada, California								
Medium, emulsive	1.75	—	—	40.7	9.1	12.5	100	100
Light, emulsive	1.75	—	0.5	30.3	3.8	1.2	56	13
Light, emulsive	1.75	1.0	—	39.3	2.7	3.4	31	28
Light, emulsion	2.0	1.0	—	32.2	3.0	2.5	42	25
—	—	3.0	—	49.2	18.6	0.3	169	2
Grove B, Chatsworth, California								
Medium, emulsion	2.0	—	—	47.1	11.0	9.6	100	100
Light, emulsion	2.0	—	0.5	37.5	3.8	2.5	43	33
Light, emulsion	2.0	1.0	—	57.9	4.8	2.5	35	21
Medium, emulsion	2.0	1.0	—	47.1	5.4	0.1	49	1
—	—	3.0	—	28.6	12.3	0.1	184	2

[a] Corrected relative infestation (Ebeling 1947); 100 = results with medium grade oil at concentrations of 1.75 and 2.0 gallons per 100 gallons.

Table 6.—Relative effectiveness of schedules of semiannual applications of malathion, parathion, and petroleum oil for control of California red scale on Eureka lemon trees at Riverside, California, as indicated by percentage of fruits and terminal green wood infested with live adult female scale before and after spray applications of November 29, 1951, and April 8, 1952.

Treatment		Amount per 100 Gallons	Application	Count Unit	Per Cent Units Infested				
No.	Material				11/2/51	3/5/52	7/7/52	10/17/52	C.R.I.[a]
1	Petroleum oil, medium-grade (emulsive)	1.75 gal.	Fall and Spring	Fruit	93.3	4.2	—	67.5	100
				Green wood	96.7	0.4	5.8	57.1	100
2	Petroleum oil, medium-grade (emulsive)	1.75 gal.	Fall	Fruit	98.3	5.0	—	72.9	103
	Malathion 25% WP	2.0 lb.	Spring	Green wood	98.8	0.0	7.1	58.8	102
3	Malathion 25% WP	2.0 lb.	Fall	Fruit	96.7	35.4	—	87.5	165
	Petroleum oil, medium-grade (emulsive)	1.75 gal.	Spring	Green wood	96.3	21.3	7.9	77.5	169
4	Malathion 25% WP	2.0 lb.	Fall and Spring	Fruit	96.7	39.2	—	95.8	182
				Green wood	96.3	19.2	21.3	94.2	214
5	Malathion 25% WP	3.0 lb.	Fall and Spring	Fruit	98.8	39.6	—	92.9	175
				Green wood	99.2	9.6	16.3	87.5	175
6	Malathion 25% WP	4.0 lb.	Fall and Spring	Fruit	97.1	35.4	—	85.4	162
				Green wood	97.5	6.7	13.8	80.8	159
7	Malathion 25% WP	2.0 lb.	Fall	Fruit	97.5	47.1	—	57.9	140
	Parathion 25% WP	2.0 lb.	Spring	Green wood	97.5	27.9	5.8	46.3	125
8	Parathion 25% WP	2.0 lb.	Fall	Fruit	99.2	10.8	—	67.9	103
	Malathion 25% WP	2.0 lb.	Spring	Green wood	98.8	7.5	7.5	65.8	125
9	Parathion 25% WP	2.0 lb.	Fall and Spring	Fruit	98.3	5.0	—	55.8	80
				Green wood	97.9	2.9	7.5	43.8	85

[a] Corrected relative infestation (Ebeling 1947); average of all posttreatment counts; 100 = results with medium-grade oil at a concentration of 1.75 gallons per 100 gallons.

spray schedules involving both spring and fall treatments, as required for the control of California red scale on lemons in certain critical areas. Results of a test based on a variety of schedules involving such fall and spring applications of malathion used alone and with petroleum oils or parathion are given in table 6.

Information accruing from studies by Elmer & Ewart (1954) concerning the favorable control of soft scale obtainable with malathion or malathion-parathion mixtures also suggested the possibility of using malathion at reduced volumes in closely sequenced applications. The results of such evaluations are presented in table 7.

DISCUSSION.—Evaluation of results of field tests for the control of California red scale may be undertaken in several ways. All aerial parts of the tree may be infested, and population counts may be taken on the larger branches or twigs, on the terminal twig growth, on leaves, or on fruit. Counts made shortly after treatment may often be made profitably on any of these tree units, since the information accumulated might suggest relative susceptibilities of the scale infesting specific tree areas and/or indicate the importance of surface characteristics of the tree in relation to the physical properties of the chemical treatment. However, such counts are frequently inadequate or misleading because of (1) the difficulties encountered in making critical determinations of individual scale mortalities within a relatively short period after application; (2) the failure of such counts to reflect the number of crawlers produced and successfully settling after the application but prior to the eventual demise of the female scales, whether due to treatment effects or natural causes; and (3) the possibilities of such counts not indicating differential kills of scale in various stages of development and/or on different tree substrates unless a suitably comprehensive but prohibitively time-consuming count is made.

Misinterpretations of results which might be fostered by restricting judgments to a single count completed shortly after the application of treatments can be largely avoided by delaying the initial posttreatment count until 4 to 6 months have elapsed, and by supplementing such a count with additional posttreatment counts extending preferably to periods approximately 1 year after the initiation of treatments. In effect, these latter counts reflect results in the most practical sense, since growers evaluate the effectiveness of their scale control programs on a season-to-season basis, even in situations where a schedule of two treatments is required annually.

The underlying significance of evaluating California red scale control treatments approximately 1 year after the initiation of the treatment is further linked to considerations such as the cropping characteristics of citrus varieties, the stage of fruit development at the time of application, the seasonal tolerance of the host to chemical treatments, and the completion of a full climatic cycle. The development of as many as 3 or $3\frac{1}{2}$ scale generations during such a period (Dickson & Lindgren 1947) is extremely helpful in permitting the scale to become established in portions of the tree in which the population may have been virtually eliminated by the treatment.

Table 7.—Relative effectiveness of single and multiple spray-blower applications of malathion and parathion used alone or in combination and single full-coverage applications of parathion and petroleum oil for control of California red scale on navel orange trees at Chatsworth, California, as indicated by percentage of fruits and terminal green wood infested with live adult female scale before and after spray applications.

Material/100 Gallons										
Pounds		Gallons	Spray Application (Gallons/Acre)			Per Cent Units Infested				
Malathion 25% WP	Parathion 25% WP	Petroleum Oil[b]	8/22/52	9/24/52	Scale-Count Unit	8/20/52	2/18/53	9/4/53	10/15/53	C.R.I.[a]
2.5	—	—	400	400	Fruit	15.3	9.7	0.3	7.7	128
					Green wood	36.2	1.2	0.0	6.9	372
1.25	1.25	—	400	400	Fruit	21.2	4.4	0.3	5.6	54
					Green wood	51.6	0.9	1.2	9.4	371
—	2.5	—	400	400	Fruit	49.4	7.8	0.6	4.7	29
					Green wood	81.6	0.3	1.9	8.1	210
2.5	—	—	800	—	Fruit	50.3	21.9	2.2	6.2	66
					Green wood	83.4	0.9	3.4	9.7	279
1.25	1.25	—	800	—	Fruit	40.6	7.5	0.3	5.6	36
					Green wood	61.6	0.0	0.0	6.9	186
—	2.5	—	800	—	Fruit	49.4	20.3	0.3	2.8	52
					Green wood	69.1	0.0	0.6	7.5	195
—	2.5	—	2400[c]	—	Fruit	15.9	1.6	0.0	0.3	13
					Green wood	33.1	0.0	0.0	1.9	95
—	—	1.75	2400[c]	—	Fruit	12.8	8.1	0.3	3.2	100
					Green wood	31.6	0.0	0.3	1.6	100

[a] Corrected relative infestation (Ebeling 1947); average of all posttreatment counts; 100 = results with light-medium oil at a concentration of 1.75 gallons per 100 gallons.
[b] Light-medium grade (emulsive) oil.
[c] Full-coverage spray applied with conventional citrus spray equipment.

On the other hand, the longer-period observations may be misleading as to the extent to which scale from external sources may infest trees in the treated plot area. To minimize such influences test plots are normally established in localities remote both from infested citrus properties not receiving adequate scale control treatments and from windbreaks or other host plants known to be infested. Reinfestation from plots within the test area which receive ineffective treatments is largely avoided by re-treating such plots with standard treatments prior to the termination of the test.

Fruits are considered particularly desirable count units in evaluating treatments for the control of California red scale since they are very definitive tree units, are normally available throughout the year (except navel oranges), and at the time of terminal counts reflect the degree of survival in all parts of the tree because of the tendency for scale crawlers to move to the peripheral parts of the tree and to select sites on fruits in such areas upon which to settle. Although it is feasible and often desirable to count the actual number of scales found on a specific tree unit or on a random selection of units, the evaluation of a treatment may be strongly biased by the presence of a number of scales on a single fruit, with the virtual absence of scales on the remaining fruits in the random sample. Such an incidence of scale on a single count unit might result from inadequate spray coverage in a localized part of the tree or from other factors independent of treatment effects. For that reason it seemed desirable to minimize the influence of individual count units on the total evaluation of a treatment, and the data presented in this paper are largely based upon the percentage of the units in the random sample infested with live adult female scale. Auxiliary counts made on count units other than fruit are included, and in a single instance (Table 1) counts of the actual number of scales found are reported.

Grove selections for the tests were based primarily on population density of the California red scale and secondarily, insofar as possible, on the uniformity of the infestation level among trees. However, differences in the initial population levels normally existed from tree to tree and from plot to plot and since it may be assumed that the number of scales initially surviving treatment reflects the magnitude of the pretreatment population as well as the efficacy of the treatment, differences in the pretreatment population density may have a notable influence on the ultimate posttreatment population density. The resultant difficulty in interpreting results based on the posttreatment data alone may be partially resolved by utilizing the equation $x = 100(ab'/a'b)$, which Ebeling (1947) suggested and termed the "corrected relative infestation" (C.R.I.). In this equation a and a' represent, respectively, pretreatment and posttreatment counts for the standard treatment, and b and b' represent similar counts for any given treatment of the experimental series. The C.R.I. values express the change in population density caused by each of the various treat-

ments in an experiment in terms of the change in one of the treatments, preferably a standard or reference treatment. Within the limits of mathematical restrictiveness the equation applies a correction factor for the difference in the pretreatment infestation levels of the various plots in a test, and these values often facilitate comparison of the treatments.

The determination of dosage requirements of malathion for the control of California red scale was attempted in several of the tests, but finite limitations were not indicated. In table 1 the data indicate slight reductions in the scale infestation levels after 10 months at dosage rates of 2 or 4 pounds of the 25 per cent wettable powder per 100 gallons. With each succeeding year, this trend has become more apparent, but it is particularly significant to note the gradual reduction of population levels with the continued annual applications of the two lower dosages (0.5 and 1.0 pound). Although infestations on leaves and on terminal green wood were lower initially than those on fruit, trends over the test period were similar to those shown on fruit. Because of the limited number of trees available in the grove area used in this test the experimental design consisted of eight single-tree replicates of each treatment, and the influence of drift was minimized only to the extent that applications were made during periods of minimal air movement. This circumstance may account in part for the relatively effective control provided by the lower dosages of malathion and by the petroleum oil spray. However, more typical results with the use of the latter material are shown in table 4, and it may be noted that even in this 2-year period of observation the lowest dosages of malathion were increasingly effective with repeated applications.

Although confirming evidence is not as yet available, the data in tables 1 and 4, and other field observations, suggest that malathion may be more effective in killing scale on leaf and bark surfaces than on fruit surfaces, in contrast to petroleum oil, which Ebeling (1932) found to give the highest kills on fruit surfaces. The removal, at harvest, of fruit as the principal reservoir of scale surviving malathion treatments would lend an advantage to such a material. An additional factor is the tendency of crawlers produced by female scale located on fruit to settle on the same fruit, whereas crawlers produced by female scale on other tree surfaces, particularly on bark surfaces, may move greater distances to find a suitable spot for settling, and often move onto fruits.

The tendency toward an improved degree of control on oranges at dosages in excess of 2 pounds of the 25 per cent wettable powder per 100 gallons spray is further shown by the data presented in tables 3 and 4. On lemons, fall and spring applications of 3 pounds of the 25 per cent wettable powder provided excellent control of moderate scale infestations (Table 5), but similar applications, even at a dosage of 4 pounds of the 25 per cent wettable powder, were ineffective against an extremely heavy infestation (Table 6). Such dosage levels are currently impracticable and suggest the advisability of using malathion only against lower infestation levels and at lower dosages.

The timing of malathion applications appears to be important, as single applications on oranges during the late summer and fall periods (Tables 1 and 4) were initially less effective, even at accelerated dosage levels, than applications made during the spring and early summer periods at lower dosage levels (Tables 2 and 3). Applications made at 2-month intervals during the spring and early summer period indicated some advantage, even within that relatively short period of the season, in making applications during the immediate postbloom period (Table 2). This observation parallels those made earlier in field tests with parathion (Carman & Ewart 1950).

As also shown in table 2, double applications of low dosages of malathion at 2- or 4-month intervals during the spring and early summer form highly effective treatment schedules. While largely impracticable on the basis of use for California red scale control alone, such treatment schedules applied with critical regard to timing for the control of another pest and/or with the inclusion of other materials might be coordinated to provide, in addition, control of other citrus pests, the correction of nutritional deficiencies, or the utilization of growth regulator materials. In such treatment schedules the dosage of malathion did not appear to be critical within the range used, and all treatments appeared to be more effective than a petroleum oil spray applied on July 15.

The indications that malathion applications were not usually as effective as petroleum oil or parathion treatments, unless dosages in excess of the present competitive amounts were used and were applied during the most favorable periods of the season, prompted investigations of the effectiveness of this material when used in combination with parathion or petroleum oils.

Combinations with parathion (Table 3) were of additional interest because, as Elmer & Ewart (1954) have reported, the mixture of these materials provides more effective control of the soft scale than malathion used by itself. Earlier work (Bartlett & Ewart 1951, Elmer *et al.* 1951) had shown the use of parathion to be conducive to the posttreatment increase of soft scale populations. The data presented in table 3 demonstrate effective control of California red scale with all treatments, possibly due in part to the time of application, even though the mature and highly infested fruit crop present at the time of application was not picked until late the following July.

The average C.R.I. values (Table 3) of the treatments with 0.5, 0.75, and 1.0 pound of 25 per cent malathion wettable powder, with uniformly varied amounts of parathion per 100 gallons, are 246.0, 190.0, and 127.7, respectively. The average C.R.I. values of the treatments with 0.5, 0.75, and 1.0 pound of 25 per cent parathion wettable powder, with uniformly varied amounts of malathion per 100 gallons, are 246.7, 163.3, and 153.7, respectively. These comparisons would suggest, within the limits of dosage variations included in the test, the use of 1 pound per 100 gallons of each 25 per cent wettable powder, with the possibility that accelerated dosages of malathion rather than of parathion might be most helpful if larger amounts were to be used. Elmer & Ewart (1954) concluded that the most effective combination for the control of soft scale consisted of 1.5 pounds of 25 per cent malathion wettable powder plus 0.5 pound of 25 per cent parathion wettable powder per 100 gallons.

Combination treatments of malathion and petroleum

oils were of particular interest because of the known advantages accruing from the use of oil for the control of such citrus pests as citrus red mite, *Metatetranychus citri* (McG.), citrus bud mite, *Aceria sheldoni* (Ewing), black scale, *Saissetia oleae* (Bern.), and others; as well as because of the possibility that the combination might provide more effective control of California red scale than either oil or malathion used independently. The initial trials shown in table 4 were extended over a 2-year period, with annual applications.

In all cases the inclusion of 25 per cent malathion wettable powder in petroleum oil sprays increased the effectiveness of the spray in control of California red scale. Although the addition of even 0.5 pound of the malathion formulation per 100 gallons was notably effective, the use of greater amounts would be indicated insofar as the cost is warranted. Reducing the amount of petroleum oil from 1.75 to 1.25 and 0.75 gallons per 100 gallons, with a uniform inclusion of 1.0 pound 25 per cent malathion wettable powder, decreased effectiveness initially but appeared less limiting with repeated use. The possibilities of reducing the amounts of petroleum oil and accelerating the dosages of malathion are currently being investigated, particularly in regard to the effects on the control of other citrus pests, such as those named above.

The grade of petroleum oil within the range of light, light-medium, and medium did not appear to be limiting with reference to California red scale control, but the use of light-grade oil may be inadequate for the control of citrus bud mite or other pests even with the malathion inclusions. The type of petroleum oil formulation used did not appear to affect the degree of control, but tests are being made currently to determine whether or not comparable results are obtainable with the inclusion of malathion either as a wettable powder or as an emulsifiable concentrate formulation. As previously noted, the repetitive use of malathion by itself gives increasingly effective results, in contrast to the repetitive use of petroleum oil sprays. Such evidence suggests the advantage of using malathion initially against lower infestation levels and continuing its use on an annual spray schedule basis. In effect, this emphasizes the use of malathion as a treatment in anticipation of population increases which will result if treatment is delayed, rather than as a treatment to be used after the scale population density has become critical.

Further evidence of the effectiveness of petroleum oil sprays fortified with malathion is shown in table 5, in which the data accruing from parallel tests on lemons are summarized. Both groves are located in areas in which two annual treatments are usually required for California red scale control. The accumulative effectiveness of malathion applied by itself is again demonstrated, although on the basis of consecutive fall and spring applications on lemons heavily infested with California red scale (Table 6) such results were not obtainable.

The use of kerosene in combination with malathion, in lieu of heavier grades of petroleum oil, provided favorable control of California red scale when applied during the postbloom period, but such combinations did not appear to be more effective than malathion or parathion used alone in amounts which would be competitive in material costs with those of the combination treatments (Table 3).

When malathion was used against an extremely heavy infestation of California red scale, as evidenced by the almost complete infestation of fruit and, particularly, of green terminal wood on Eureka lemon trees (Table 6), certain characteristics were apparent. The most obvious of these is that malathion used by itself is not as effective under such conditions as petroleum oil or parathion on the basis of effects on initial populations. While some increase in effectiveness was observable with increments in dosages, this factor was not limiting within the dosage range employed. In all cases, treatment schedules in which malathion was applied in the spring were more effective than those in which the treatments were reversed in sequence in the schedule and the malathion applied in the initial fall treatment. However, such spring applications of malathion were used against apparently reduced population levels. Direct comparisons of the effectiveness of the treatment schedules on fruit and on green terminal wood are largely invalidated by considerations of the time lapses between applications and posttreatment counts, although the posttreatment counts completed on March 5, 1952, may be significant in this regard since they were completed prior to any spring picking.

Control provided by multiple applications of malathion at reduced volumes per acre, using spray-blower equipment of the Master Fan type, was poorer than that provided by a single application utilizing the same total volume per acre (Table 7). More definitive studies of the potential use of malathion when applied with spray-blower equipment are in progress, even though the use of parathion in this manner was shown in this field trial to be more effective. However, closely sequenced applications of parathion may be conducive to the posttreatment build-up of soft scale (Elmer *et al.* 1951) and can only be suggested for use in combination with malathion.

In the course of these studies no adverse tree effects have been observed to be associated with applications of malathion. However, it remains possible that acute phytotoxicities may be encountered under certain conditions of use, or that chronic phytotoxicities may result from long-continued usage. In direct comparisons of malathion and petroleum oil combinations with petroleum oil used alone, the evidences of posttreatment tree reaction have been very similar, in contrast to the earlier experience with parathion and petroleum oil combinations (Carman & Ewart 1950) in which increased phytotoxicity was observed. The use of malathion has not appeared to predispose navel oranges to the incidence of water spot during periods of prolonged wetting.

Although malathion applications of the type reported in this paper did not constitute adequate control treatments for certain other pests co-existing in the treatment areas with the California red scale, no instances were encountered of a posttreatment build-up of populations of any of these insects or mites beyond normal expectancies in the absence of a specific control treatment.

SUMMARY AND CONCLUSIONS.—Results of preliminary field tests with malathion for the control of California red scale on citrus in California are reported. On the basis of earlier indications of malathion's effectiveness, tests were made of dosage requirements, approximate timing

of applications, value of treatment schedules of closely sequenced applications, effectiveness of combinations with other materials, and of the usefulness of malathion as a treatment for inclusion in annual spray schedules. In these connections, opportunities were afforded to apply malathion with an oscillating boom sprayer and with spray-blower equipment, as well as manually with conventional citrus sprayer units.

It is concluded (1) that malathion can be used effectively for the control of light and moderate infestations of California red scale on citrus in California, provided that dosages generally in excess of 2 pounds 25 per cent wettable powder per 100 gallons are applied during the immediate postbloom period; (2) that it can be used effectively in combination with either parathion or various grades of petroleum oil; and (3) that as used in these studies it is relatively non-phytotoxic and is not instrumental in contributing to the posttreatment increase of insect or mite populations against which it is largely ineffective.

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Effect of Formulations and Methods of Application of Insecticides on the Control of Wireworms on Sweet Potatoes[1]

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Wireworms have been reported by Cockerham & Deen (1936) and Griffin & Eden (1953) to be one of the most important groups of soil insect pests of sweet potatoes in Alabama. Injury occurs in the form of feeding punctures, which deface the surface of the sweet potato and lower the market value. These punctures also serve as ports of entry for soil and storage rots.

The economic importance of wireworms in Alabama was first recognized by Cockerham & Deen (1936) in 1927 when they found sweet potatoes being damaged severely by the Gulf wireworm, *Conoderus amplicollis* (Gyll.). Griffin & Eden (1953) reported satisfactory control of this species with dilute dusts of aldrin, dieldrin, heptachlor, and lindane applied to the soil as insecticide-fertilizer mixtures at a rate of 2 pounds of technical material per acre. Merrill (1952) obtained effective control of wireworms attacking potato tubers with 1.5 pounds of aldrin, dieldrin, or lindane dusts. The materials were applied broadcast to the soil surface and cultivated in immediately. Dupree *et al.* (1955) obtained good control of a wireworm, *Drasterius dorsalis* (Say), and two species of flea beetles on sweet potatoes with heptachlor, lindane, BHC, or aldrin in fertilizer mixtures. Floyd (1955) obtained excellent control of soil insects in sweet potatoes with 2 pounds of aldrin per acre.

Experiments were conducted in Alabama in 1953 and 1954 to test the effects of various formulations and methods of application of insecticides on the control of wireworms attacking sweet potatoes. The results of these experiments are herein reported.

PROCEDURE.—Field tests were conducted at Cullman, Clanton, and Ashford in northern, central, and southern Alabama, respectively. The tests involved the introduction by various methods of three formulations of aldrin, dieldrin, heptachlor, and lindane into the soil. Aldrin was used at all three locations and the remaining compounds were tested with aldrin as follows: dieldrin at Cullman, heptachlor at Clanton, and lindane at Ashford. The formulations tested were dilute dusts, granules, and emulsions. The dusts and granules were applied to the soil as insecticide-fertilizer mixtures and as broadcast treatments. These applications were made 1 to 2 weeks prior to setting of the sweet potato plants. Emulsions were

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Table 1.—Wireworm injury and yield of sweet potatoes following various insecticidal treatments, Ashford, 1953–1954.

Treatment	Per Cent Undamaged Sweet Potatoes			Mean Bushels per Acre[2]
	1953	1954	Two-Year Mean[1]	
Untreated Check	69.5	60.8	54.11	93.2
Aldrin				
Granules broadcast	80.5	100.0	77.85	72.1
Granules in fertilizer	73.6	99.2	74.28	72.7
Dust broadcast	94.9	99.3	82.34	98.0
Dust in fertilizer	91.5	99.0	80.63	82.5
Emulsion in trans. water	85.5	98.5	77.20	87.8
Lindane				
Granules broadcast	77.6	97.9	74.55	62.3
Granules in fertilizer	92.4	98.5	81.46	95.7
Dust broadcast	83.5	98.5	76.42	80.3
Dust in fertilizer	76.0	96.4	71.82	59.0
Emulsion in trans. water	64.7	100.0	73.74	47.0
L.S.D. at 5% level	—	—	15.81	44.9
L.S.D. at 1% level	—	—	21.00	—

[1] Transformed to angles for statistical treatment.
[2] These yields include wireworm damaged, but otherwise marketable sweet potatoes.

applied to the soil in the transplant water at the time of plant setting. The rate of application in all cases was 2 pounds of technical insecticide per acre.

Insecticide-fertilizer mixtures were applied as row treatments to the open furrow. Broadcast treatments of insecticides were applied by spreading the materials over the soil surface by hand before closing the furrow to form the plant bed.

The emulsifiable concentrates were mixed with the transplant water and applied by hand or mechanically to the soil around the sweet potato plants in the setting and watering process. Sweet potato slips were set by a tractor-drawn mechanical planter.

The experiments at each location involved 11 treatments, including a non-treated check. The treatments were replicated four times in randomized blocks. The plots consisted of five rows, 3½ feet apart and 30 feet long.

Sweet potatoes in all experiments were harvested 150 to 180 days after insecticidal treatments were applied to the soil. The three inside rows of each plot were harvested for the purpose of computing yield data and measuring wireworm injury. The sweet potatoes were separated into two grades, namely marketable and cull. Wireworm injury was not used as a criterion in grading. Wireworm injury and yield data were determined from marketable sweet potatoes only. Marketable roots were inspected individually for wireworm injury and placed into four categories according to the degree of injury. These were clean or no injury, and light, moderate, and heavy injury. These categories were used as a basis for evaluating the effectiveness of insecticidal treatments.

In addition to the control tests, a soil sampling program was conducted in 1954 to determine the prevalent species of wireworm occurring in the test areas.

RESULTS.—The growing seasons of 1953 and 1954 were extremely dry in Alabama and were poor years for sweet potato production. Yields in general were low and wireworm infestations in most areas were light. All wireworms collected from the test areas were found to belong to the genus *Conoderus*.

Results of the control experiments are given in tables 1, 2, and 3. At Ashford (Table 1) where various formulations and methods of application of aldrin and lindane were tested, wireworm injury was relatively light. All chemical treatments, however, were effective in significantly reducing this injury. No differences in root injury occurred between treated plots due to insecticide, type of formulation, or method of application.

Wireworm injury was also light at the Clanton location (Table 2). Nine of the ten insecticidal treatments resulted in increased percentages of clean sweet potatoes. Plots receiving applications of heptachlor granules in the fertilizer failed to show a significant increase over the untreated check. With the exception of this treatment, all insecticidal treatments were equally effective in wireworm control, regardless of the type of formulation or method of application employed.

At Cullman (Table 3) where dieldrin was tested with aldrin, damage to sweet potatoes by wireworms was severe. An average of only 21 per cent of the sweet potatoes harvested from untreated check areas was clas-

Table 2.—Wireworm injury and yield of sweet potatoes following various insecticidal treatments, Clanton, 1953–1954.

Treatment	Per Cent Undamaged Sweet Potatoes			Mean Bushels per Acre[2]
	1953	1954	Two-Year Mean[1]	
Untreated Check	82.6	59.1	58.19	84.1
Aldrin				
Granules broadcast	92.5	94.6	76.87	74.3
Granules in fertilizer	91.7	93.0	74.23	81.4
Dust broadcast	90.3	92.4	77.11	72.4
Dust in fertilizer	95.5	92.9	76.99	72.5
Emulsion in trans. water	96.0	94.6	77.64	97.2
Heptachlor				
Granules broadcast	94.7	85.5	74.73	66.5
Granules in fertilizer	90.3	76.1	67.25	73.1
Dust broadcast	87.0	91.7	73.57	72.8
Dust in fertilizer	93.8	93.6	77.39	80.1
Emulsion in trans. water	95.4	93.8	79.79	80.4
L.S.D. at 5% level	—	—	11.98	None
at 1% level	—	—	15.90	None

[1] Transformed to angles for statistical treatment.
[2] These yields include wireworm damaged, but otherwise marketable sweet potatoes.

Table 3.—Wireworm injury and yield of sweet potatoes following various insecticidal treatments, Cullman, 1953–1954.

Treatment	Per Cent Undamaged Sweet Potatoes			Mean Bushels per Acre[2]
	1953	1954	Two-Year Mean[1]	
Untreated Check	26.5	15.5	26.64	143.5
Aldrin				
Granules broadcast	78.8	42.8	52.23	133.8
Granules in fertilizer	64.5	52.7	50.32	147.5
Dust broadcast	71.4	40.9	48.89	141.3
Dust in fertilizer	55.3	33.6	41.57	122.2
Emulsion in trans. water	59.1	18.7	37.68	122.3
Dieldrin				
Granules broadcast	82.6	50.2	55.44	151.9
Granules in fertilizer	65.9	44.4	48.20	138.9
Dust broadcast	71.8	44.7	50.11	131.6
Dust in fertilizer	72.1	36.8	47.74	133.7
Emulsion in trans. water	68.9	25.8	43.46	133.5
L.S.D. at 5% level	—	—	12.06	None
at 1% level	—	—	16.01	None

[1] Transformed to angles for statistical treatment.
[2] These yields include wireworm damaged, but otherwise marketable sweet potatoes.

sified as damage-free. Broadcast applications of granulated formulations appeared to be slightly more effective than all other treatments. Applications of emulsions in the transplant water were the least effective. Treatments of aldrin emulsion in the transplant water failed to significantly reduce wireworm injury to sweet potatoes when compared with the untreated check. Broadcast applications of aldrin and dieldrin granules, dieldrin dust, and insecticide-fertilizer mixtures of aldrin granules were significantly more effective than aldrin emulsion applied in the transplant water.

The yield of sweet potatoes was somewhat variable between treatments at all locations. At the 5 per cent level of probability, the yield of plots receiving treatments of lindane emulsion in the transplant water at Ashford (Table 1) was significantly lower than that of the untreated check. No other significant differences in yields were exhibited as a result of treatments at any other location.

SUMMARY.—Wireworms are important soil insect pests of sweet potatoes in Alabama. Their feeding punctures mar the appearance of the sweet potato and lower the market value. Experiments were conducted in 1953 and 1954 to determine the effect of various formulations and methods of application of aldrin, dieldrin, heptachlor, and lindane on the control of wireworms attacking sweet potatoes. Formulations used were dilute dusts, granules, and emulsions. The dusts and granules were applied as insecticide-fertilizer mixtures and as broadcast treatments prior to setting of plants. Emulsions were applied in the transplant water at planting time. The rate of application was 2 pounds of technical material per acre.

All sweet potatoes were harvested 150 to 180 days after treatment. Wireworm injury and yield data were based on marketable roots only.

At the Ashford and Clanton locations where wireworm injury was light, all formulations and methods of application tested were equal in effectiveness. At Cullman where injury was severe, broadcast applications of granulated formulations appeared to be slightly more effective than all other treatments. Applications of emulsifiable concentrates in the transplant water were the least effective. Aldrin emulsion in the transplant water failed to significantly reduce wireworm injury. The yield of plots receiving lindane emulsion in the transplant water at Ashford was significantly lower than that of the untreated check plots.

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Granulated Insecticides for European Corn Borer Control[1]

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According to Farrar (1953), granulated formulations of certain insecticides controlled a heavy infestation of the corn earworm in late field corn in the whorl stage. Since young larvae of the first-brood European corn borer, *Pyrausta nubilalis* (Hbn.), feed primarily in the whorl leaves of corn, experiments were conducted in 1953 and 1954 to find out if similar materials would control them. Early applications showed that the granules tended to adhere temporarily to the corn leaves and then to roll slowly into the whorls and leaf axils. In view of these observations and the fact that young second-brood borers do their early feeding in the leaf axils, granulated formulations were also tested for control of second-brood borers.

All experiments were carried out on the Iowa State College experimental farm at Ankeny, Iowa. In the hope of securing a heavy natural infestation of borers, early-planted fields were chosen for tests with the first brood and late-planted fields for tests with the second brood. This scheme worked very well.

MATERIALS.—The insecticides tested in granulated formulations were DDT, heptachlor, EPN, and malathion. The granules were prepared with two kinds of carriers, attapulgite and tobacco-base.

The type of attapulgite used in 1953 was not known, as each formulation was obtained from a different company,[5] but in 1954 RVM-AA 30/60-mesh Attaclay was used in preparing all the granules with this carrier.[6] All the tobacco-base granules were formulated by one company.[7]

EQUIPMENT.—Early applications were made with a self-feeding power duster, but this proved to be an ineffective method. Because of their physical structure, the tobacco-base formulations tended to bridge at the feeding openings and cause uneven flow. The attapulgite particles packed in the hopper bottom. The mechanical agitator caused some breakdown of the particles. This packing was serious enough to break the agitator and the feed belt. The granules, being carried in the relatively strong air stream created by the duster fan, bounced off the corn

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[2] Entomology Research Branch, Agr. Res. Serv., U.S.D.A.

[3] Iowa State College.

[4] Agricultural Engineering Research Branch, Agr. Res. Serv., U.S.D.A.

[5] DDT was obtained from the Niagara Chemical Division, Food Machinery and Chemical Corp.; heptachlor from the Velsicol Corp.; and EPN from E. I. du Pont de Nemours and Co.

[6] Formulated by the Mackwin Co., Winona, Minn. Attaclay is a product of the Attapulgus Minerals & Chemical Corp.

[7] Black Leaf Products Division of Virginia-Carolina Chemical Corp.

leaves rather than rolling into the whorls of the plants.

To overcome these difficulties, a front-mounted, fluted-feed grass seeder, powered by the tractor battery and an automotive generator, functioning as a direct-current motor, was used in 1953. The front mounting gave sufficient clearance for the application for first-brood control, and a fairly uniform flow of granules was directed into the whorls.

Residue analyses showed that the seeder-type duster did a better job of putting the insecticides where they were needed than the power duster. This equipment was adapted for experimental plot work. If field experiments continue to show excellent borer control, then equipment for applying these granules on a large scale must be developed. Since the power duster was designed for application to six rows simultaneously and the seeder-

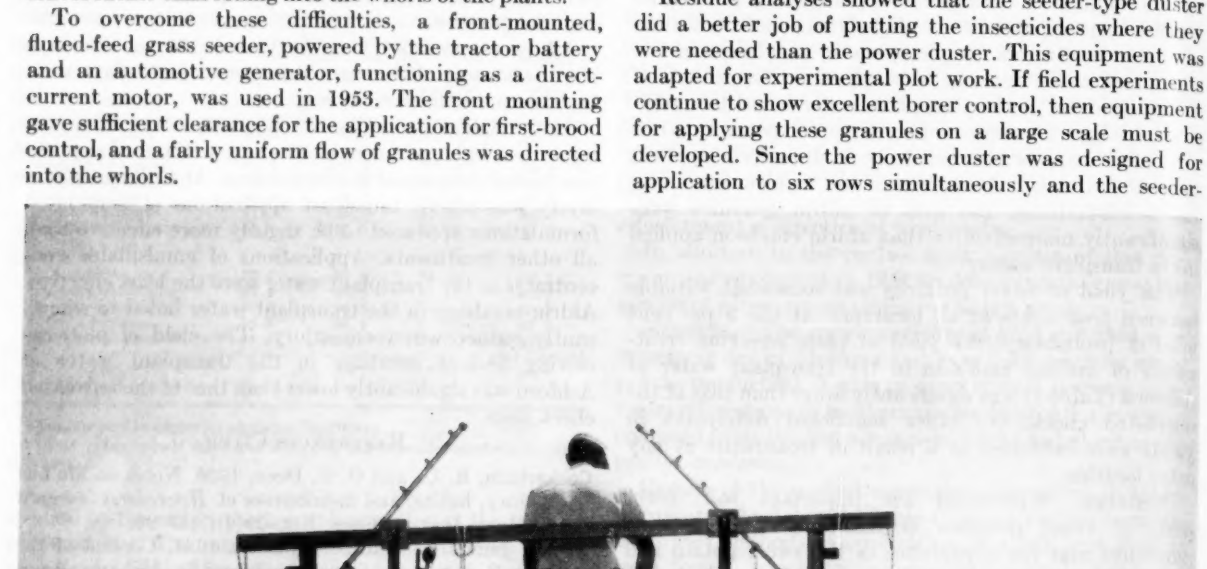

FIG. 1.—Rear view of experimental seeder-type duster used in the 1954 experiments. Mounted on the framework of the high-clearance sprayer in this manner, this duster can be used to apply the granular material for second- as well as first-brood borer control.

A different type of seeder was used in 1954. This was a 5-foot experimental grass seeder,[8] which used a double-chain metering system with offset metering holes in the false bottom to insure uniform flow. By utilizing the converted generator and a 6-volt storage battery for power and mounting the seeder box onto the framework of a high-clearance sprayer, sufficient clearance was obtained to apply granules for control of both first- and second-brood borers. Figure 1 shows this seeder as it was used against the first brood. By closing all box openings except the three that were directly over each row, it was possible to concentrate the applications over the centers of the corn plants.

type duster to only two, variation in plot size within an experiment was sometimes necessary.

GENERAL PROCEDURE.—The design of each experiment was a randomized complete block in which each treatment, including an untreated control, was replicated four times. With exceptions noted, all the granulated formulations were applied at the rate of 20 pounds per acre. Specific procedures are presented in the discussion of each experiment.

In order to determine the effectiveness of insecticide applications in relation to the amount of borer egg laying

[8] Furnished by the Century Engineering Corp.

in some of the 1954 experiments, 10 plants in the untreated plots were randomly tagged for observation as to when egg masses were first found in the field. On each Monday, Wednesday, and Friday records were taken on the numbers of new, missing, and hatched egg masses. A felt-tip fountain pen filled with indelible ink was used to mark the locations of the egg masses.

CONTROL OF THE FIRST BROOD.—The effectiveness of first-brood experiments was determined by taking 20 plants at random from each plot and dissecting them when fifth-instar larvae began to appear in abundance. Records were made of the number of larvae in each instar and the number of tunnels in the stalks.

Experiments in 1953.—In the initial experiment granulated EPN, DDT, and heptachlor were applied to whorl-stage corn on June 26. At that time all the plants chosen for observation showed leaf feeding. As shown in table 1, there were highly significant differences between the untreated control and the treatments, but no differences between EPN, DDT, and heptachlor. It should be noted that the insecticides were not applied at the same rate. There were significantly fewer borers in plots treated with attapulgite formulations than in plots treated with tobacco formulations. Results obtained with the seeder-type duster were no better than those obtained with the power duster.

Experiments in 1954.—Two experiments for control of the first brood were carried out. One experiment was similar to the one described above in that different toxicants and carriers were compared. A DDT emulsion spray was used as a standard of comparison. The granules were applied with the seeder-type duster. All applications were made on June 30, when approximately 85 per cent of the plants showed leaf feeding.

The data obtained in this experiment are summarized in table 2. Although the experiment was started as a split-plot design, the data could not be analyzed as such since the lack of two formulations necessitated the use of substitutes.

In analyses of the DDT and heptachlor data only, there was no significant difference in the number of larvae or tunnels between the two insecticides, or between the tobacco-base and attapulgite carriers. The insecticide-carrier interaction was not significant for either tunnels or larvae. In the plots treated with the attapulgite formulations only, there was no significant difference between any of the insecticides. Even so there were nearly twice as many larvae and tunnels in the malathion plots as in any of the other plots.

Table 1.—Average numbers of first-brood European corn borer larvae and tunnels per 20 plants after treatment with granulated insecticides, 1953.

GRANULES	POUNDS OF ACTIVE INGREDIENT PER ACRE	CARRIER	DUSTER	LARVAE	TUNNELS
Untreated control	—	—	—	13.5	39.8
EPN 0.5%	1/10	Attapulgite	Power	3.0	14.0
DDT 5%	1	Attapulgite	Seeder-type	1.5	4.5
			Power	2.0	5.5
	1½	Tobacco	Seeder-type	4.3	15.5
			Power	5.0	20.3
Heptachlor 10%	2	Attapulgite	Seeder-type	3.5	12.5
			Power	4.0	9.3
	1½	Tobacco	Seeder-type	3.8	8.8
			Power	5.8	15.8

Table 2.—Average numbers of first-brood European corn borer larvae and tunnels per 20 plants after treatment with granulated insecticides with a seeder-type duster, 1954.

GRANULES	POUNDS OF ACTIVE INGREDIENT PER ACRE	CARRIER	LARVAE	TUNNELS
Controls:				
Untreated	—	—	24.5	25.3
DDT emulsion spray	1½	—	11.3	16.0
Malathion 10%	2	Attapulgite	14.8	20.5
EPN 2.5%	½	Attapulgite	8.0	11.0
DDT 7.5%	1½	Attapulgite	7.8	11.8
		Tobacco	7.3	8.8
Heptachlor 7.5%	1½	Attapulgite	8.3	12.5
		Tobacco	8.0	13.3

Although there were fewer larvae and tunnels in the DDT-sprayed plots than in the malathion plots, there were more than in any of the other plots. It was therefore concluded that there was no significant difference between emulsion sprays and granulated formulations against the first brood in 1954.

An experiment to compare the effectiveness of different dates of application of 7.5% DDT on granulated attapulgite is summarized in table 3. The numbers of both larvae and tunnels showed highly significant differences between the untreated and the treated plots. There were significant differences in the number of larvae found following the different application dates; however, there was no difference in the number of tunnels. The two sets of data showed the same general trend but there was greater variation in tunnel numbers.

As shown in figure 2, granulated DDT was applied at the time of peak egg laying and 2 and 9 days after peak egg hatching. The data in table 3 indicate that the July 7 application was made too late to reduce the borer population appreciably. Since almost 75 per cent of the eggs deposited had hatched 7 days before this last treatment, the borers were probably too deep in the whorl leaves and tissue to be reached by the granules.

On the basis of this experiment, probably the best control can be obtained with two applications of DDT granules, the first about the time of peak egg laying and the second at or just after the peak hatching. The best single-application control will probably result from treating at or just after the peak number of eggs have hatched.

CONTROL OF THE SECOND BROOD.—The plants in plots treated for second-brood borer control were dissected when practically all the larvae were in the fifth instar. Consequently no record was kept of the different instars. Ten plants were taken at random from each plot and dissected.

Experiment in 1953.—In 1953 only attapulgite formu-

Table 3.—Average numbers of first-brood European corn borer larvae and tunnels per 20 plants after treatment with 7.5% granulated DDT at 20 pounds per acre on different dates, 1954.

APPLICATION DATES	LARVAE	TUNNELS
Untreated control	34.3	58.5
June 23	12.8	20.3
June 23 and 30	8.3	14.5
June 30	10.5	21.5
June 30 and July 7	6.8	16.8
July 7	25.5	37.3

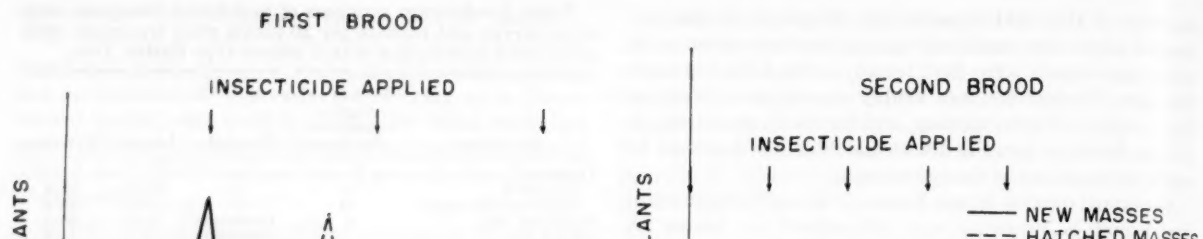

FIG. 2.—Time of insecticide applications in relation to corn borer egg laying and hatching at Ankeny, Iowa, in 1954.

lations of DDT (5%) and heptachlor (10%) were compared. A powder duster was used to apply both materials on August 12. Over 100 egg masses per 100 plants were observed on that date.

The results of this experiment, summarized in table 4, show the best control of corn borer larvae in all experiments performed with granulated insecticides. The reductions of both borers and tunnels were highly significant. DDT caused a larval reduction of almost 82 per cent and heptachlor of more than 90 per cent. Significantly fewer larvae were found in the heptachlor-treated plots than in the DDT-treated plots, but there was no difference between the numbers of tunnels. It should be remembered that the dosage of heptachlor was twice that of DDT.

Experiments in 1954.—One experiment was designed to compare the following: (1) DDT, heptachlor, EPN, and malathion; (2) the tobacco-base and attapulgite formulations of DDT and heptachlor; and (3) the power and seeder-type dusters. A shortage of 7.5% DDT on attapulgite necessitated the use of a 5% formulation, but the dosage of granules was increased to give the same amount of DDT. When the applications were made on August 4, the cumulative total egg count had reached 100 masses per 100 plants.

Table 4.—Average numbers of second-brood European corn borer larvae and tunnels per 10 plants after treatment with granulated DDT and heptachlor, 1953.

GRANULES	POUNDS OF ACTIVE INGREDIENT PER ACRE	LARVAE	TUNNELS
Untreated control	—	228.3	247.8
DDT 5%	1	42.0	74.5
Heptachlor 10%	2	22.3	44.5

Table 5 gives a summary of the results obtained. In an over-all analysis there was no significant difference in borer control between treatments, including the untreated control. However, data from the DDT and heptachlor plots showed a significant reduction in the number of larvae below that in the untreated plots. On this basis there was no difference in effectiveness between DDT and heptachlor. No significant difference was found between the attapulgite and tobacco-base formulations of these materials. The power duster was apparently just as effective as the seeder-type duster.

There was no significant difference between malathion and EPN. However, the control obtained with EPN was more comparable to that obtained with DDT and heptachlor. Based on data from the malathion and EPN treatments, the seeder-type duster was significantly better than the power duster.

A second experiment was made in 1954 to determine the best time of insecticide application for second-brood

Table 5.—Average numbers of second-brood European corn borer larvae and tunnels per 10 plants after treatment with granulated insecticides, 1954.

GRANULES	POUNDS OF ACTIVE INGREDIENT PER ACRE	CARRIER	DUSTER	LARVAE	TUNNELS
Untreated control		—	—	67.8	74.0
DDT 7.5%	1½	Attapulgite	Seeder-type	27.3	34.5
			Power	58.3	68.8
5%		Tobacco-base	Seeder-type	40.8	45.3
7.5%			Power	44.0	48.3
Heptachlor 7.5%	1½	Attapulgite	Seeder-type	40.3	50.3
			Power	33.3	43.5
		Tobacco-base	Seeder-type	39.5	48.3
			Power	46.5	53.8
EPN 2.5%	½	Attapulgite	Seeder-type	25.8	31.0
			Power	54.0	65.8
Malathion 10%	2	Attapulgite	Seeder-type	42.8	58.3
			Power	61.8	72.5

Table 6.—Average numbers of second-brood European corn borer larvae and tunnels per 10 plants after treatment on different dates with granulated DDT and emulsions of malathion and DDT, 1954.[a]

Application Dates	Granulated DDT		DDT Emulsion		Malathion Emulsion	
	Larvae	Tunnels	Larvae	Tunnels	Larvae	Tunnels
July 23	20.5	26.3	38.5	44.0	62.5	77.5
July 23 and 28	25.8	36.3	37.3	47.5	76.0	90.8
July 28	21.3	29.7	41.0	51.5	73.8	85.3
July 28 and Aug. 2	25.0	33.8	34.5	44.3	68.8	89.0
Aug. 2	31.8	42.3	32.0	44.3	34.0	42.8
Aug. 2 and 7	15.8	24.5	23.3	31.3	61.8	74.0
Aug. 7	28.0	35.3	24.3	32.5	45.0	62.0
Aug. 7 and 12	29.5	42.5	23.3	32.5	73.5	88.8
Aug. 12	33.8	42.0	24.0	40.8	52.3	71.5

[a] DDT applied at 1½ pounds and malathion at 2 pounds per acre. Untreated plants had 67.8 larvae and 85.3 tunnels per 100 plants.

borer control. A 7.5% formulation of DDT on attapulgite was the only granulated insecticide used; however, emulsion sprays of DDT at 1½ pounds and malathion at 2 pounds per acre were included for comparison. The first application was made on July 23, just 3 days before the first egg laying. Applications were made at intervals of 5 days with the seeder-type duster. Both single and double applications were made.

The results obtained from this timing experiment are summarized in table 6. There were highly significant differences between the treated and the untreated plots, and also between the three insecticides, but very little difference between granulated DDT and DDT emulsion. Almost as many borers and tunnels were found in the malathion plots as in the untreated plots. Therefore, it must be concluded that malathion was ineffective for corn borer control in this experiment. The ineffectiveness of malathion may be partially explained by residue data, which show that an application of 2 pounds per acre is completely lost in 5 days.

No significant differences in larvae or tunnels were found between the different dates of application. The number of borers in the plots treated with granules was consistently low. There were fewer borers in the DDT plots sprayed after August 2. There did not seem to be any close relation between the date of spraying and the egg deposition and hatching pattern (Fig. 2). Apparently the granulated material was effective even when applied just before or at the time second-brood egg laying began.

When the first application was made on July 23, approximately 35 per cent of the plants were shedding pollen. Five days later, when the second application was made, all the plants were shedding pollen. The authors believe there is a close relation between pollen shedding and the date granulated insecticides are applied and that this must be considered if good second-brood borer control is to be obtained. Further work is needed to verify or disprove this hypothesis.

The third experiment in 1954 was on rates of application. DDT and EPN on attapulgite were applied on August 6 with the seeder-type duster. The egg count on the day of application was about 118 masses per 100 plants. Previous experiments had shown that emulsion sprays of DDT at 1½ pounds and EPN at ½ pound per acre gave good borer control. In this experiment with granules one-half and one-fourth these dosages were used. Although they caused highly significant reductions in the numbers of larvae and tunnels as compared with the untreated plots (Table 7), the reductions were not large enough to be considered effective control. Furthermore, there was no significant difference between the two rates of application of either material. DDT was not significantly better than EPN.

Table 7.—Average numbers of second-brood European corn borer larvae and tunnels per 10 plants after treatment with low dosages of DDT and EPN, 1954.

Granules	Pounds of Active Ingredient per Acre	Larvae	Tunnels
Untreated control		61.0	86.3
DDT 7.5%	0.75	43.0	62.3
	.375	43.5	64.8
EPN 2.5%	.25	51.3	77.0
	.125	43.8	61.5

Residue Analyses.—To compare the amount and location of insecticide deposits, 10 plants were taken at random from plots treated with DDT emulsion spray and DDT granules in the 1954 experiment on time of application for control of the second brood. Plants from these plots, treated on August 2, were sampled on August 5 and 6. Since the granulated material in this experiment was applied with the seeder-type duster, another set of samples was taken from similar plots that had been treated with the power duster. These plots were treated on August 4 and sampled for residue on August 9.

The scheme of plant dissections for chemical analysis is shown in figure 3. The portion of the plant below the ear leaf was discarded. The remaining portion of the plant was divided into nine primary sections—(1) the husk (the ear was discarded), (2) the leaf and stalk at the ear, (3) the first leaf and stalk above the ear, (4) the second leaf and stalk above the ear, and so on up to and including the seventh leaf above the ear. Each primary section was subdivided by cutting off the leaf about ½ inch away from the axil and then cutting across the leaf where it would normally tend to bend downward, giving (*a*) a portion of the stalk and sheath plus axil, (*b*) a leaf base, and (*c*) a leaf tip. The leaf in section (8) was usually very small and consequently not cut across the middle. The tassel and leaves that occasionally occurred above this leaf were discarded.

All analogous portions of the 10 dissected plants from each plot were pooled in ice-cream cartons, labeled, weighed, and frozen in a deep-freezer. The samples were then shipped to the Entomology Research Branch laboratory at Vincennes, Ind., for analysis. The DDT residues were recovered by extraction and analyzed by the Stiff-Castillo colorimetric method described by Fahey & Rusk (1951).

The amounts of residue found on the various plant sections are summarized in table 8. With one exception no more than the normal variations between replications were found. Samples from the fourth replication of the plots treated with the seeder-type duster had from 2 to more than 20 times the residue found on the other three replications. Therefore, the values shown for that treatment are the average of only the first three replications. This plot must have been accidentally sprayed with DDT

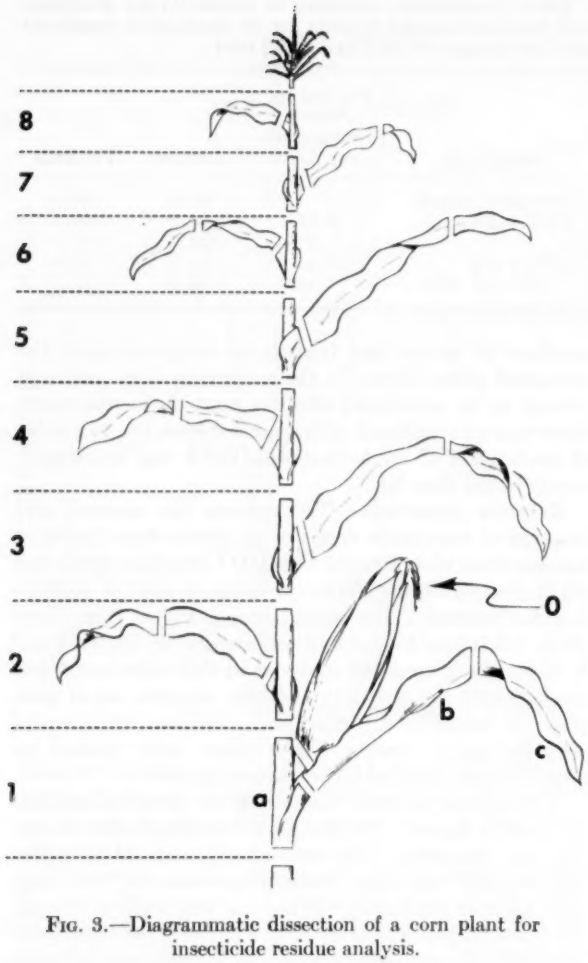

FIG. 3.—Diagrammatic dissection of a corn plant for insecticide residue analysis.

emulsion, since the amount and pattern of the residue were similar to those of a DDT emulsion.

The amount of DDT residue was much lower on the granule-treated plots than on plots sprayed with the emulsion. There was very little DDT on either the leaf bases or the leaf tips of plants taken from the granule-treated plots. Quite the opposite was true of the sprayed plots. Fahey *et al.* (1953) reported greater residue with emulsion sprays than with dusts. In the experiment reported here the insecticide deposit on leaves was reduced even more by the use of granules. Most of the particles either bounced and rolled off the leaves or rolled down the leaves into the axils. Consequently, there was little or no residue on the blades of the leaves. The toxicants accumulated in the leaf axils.

There was less residue on the leaf-axil and stalk area of plants treated with granules than on the same area of sprayed plants. As pointed out above, the rolling nature of the granules caused them to be left in the leaf axils, whereas the spray was deposited over the entire leaf-axil and stalk area. Therefore, in the leaf axil proper there was actually more residue from the granules than from sprays.

Since the leaf axil is the primary point of establishment of second-brood borers, it seems significant that there were fewer larvae in the granule-treated plots than in the sprayed plots. Decker *et al.* (1947) believed that the higher degree of borer control obtained in plots sprayed with DDT in oil than in dusted plots was due to a greater amount of residue. However, results from these experiments seem to substantiate the need for further studies on the most effective location, rather than amount, of insecticide deposits for borer control.

Table 8.—DDT residues (parts per million) on corn plants treated[a] with sprays and granules for control of second-brood European corn borers.

Plant Part[b]		Emulsion Spray	Granules: Seeder-Type Duster	Granules: Power Duster
Husk	0	4.3	1.2	3.4
Stalk and leaf axil:				
At ear leaf	1	10.0	6.4	6.4
above ear	2	16.1	8.8	5.0
	3	20.1	13.6	3.5
	4	20.2	6.0	3.0
	5	16.4	7.7	2.2
	6	10.0	7.8	1.3
	7	4.6	3.7	1.3
	8	2.7	0	1.5
Leaf base:				
Ear leaf	1	109.5	4.1	1.8
above ear	2	143.8	0	1.2
	3	168.2	1.3	0.8
	4	160.0	0.4	0.7
	5	104.2	0.4	0.6
	6	73.3	0.5	1.1
	7	21.0	0	6.9
	8	31.5	2.2	1.8
Leaf tip:				
Ear leaf	1	190.1	1.5	4.1
above ear	2	211.3	1.5	3.1
	3	289.4	1.4	2.9
	4	246.5	1.4	1.5
	5	183.4	1.4	1.2
	6	200.3	0.8	1.8
	7	69.5	0.1	7.1

[a] Rate of application 1½ pounds of DDT per acre.
[b] Numbers refer to section in figure 3.

Since cattle are often pastured on the stover in corn fields following harvest, if the fields have been treated with an insecticide it may accumulate in the body fat or be excreted in the milk. As shown in table 8, there was 100 to 200 times more residue on plants in sprayed plots than on plants treated with granules.

SUMMARY AND CONCLUSIONS.—Experiments were conducted at the Iowa State College experimental farm at Ankeny, Iowa, in 1953 and 1954 to see if granulated formulations of insecticides would control the European corn borer, *Pyrausta nubilalis* (Hbn.). A power duster and two modified grass seeders were used to apply the granules in experiments on both first- and second-brood larvae.

There was no difference between EPN, heptachlor, and DDT for first-brood borer control in 1953. Attapulgite formulations were superior to tobacco-base formulations. The seeder-type duster did not give significantly better results than the power duster, but less material was wasted and fewer mechanical breakdowns occurred.

There was no significant difference between DDT, malathion, heptachlor, and EPN or between the attapul-

gite and tobacco-base carriers for first-brood borer control in 1954.

In 1953 DDT reduced the number of second-brood borers by almost 82 per cent and heptachlor by more than 90 per cent. In 1954 heptachlor and DDT at 1½ pounds and EPN at ½ pound per acre reduced second-brood populations, but DDT and EPN at ¾ and ⅜ pounds were not effective. Attapulgite formulations were just as effective as tobacco-base. There was very little difference between DDT granules and emulsion sprays. The seeder-type duster was more effective than the power duster.

The amount of egg laying and hatching on the date the granules were applied affected the control of the first brood but not of the second brood. The best one-application control of the first brood can probably be attained with a treatment at or just after the peak of hatching.

Up to 200 times more residue was found on the leaves of sprayed plants than on those treated with granules. Sprayed plants had about twice as much residue in the leaf-axil and stalk area. More work is needed to determine the best location of the deposits with relation to second-brood borer control.

Results of these experiments indicate that granulated formulations of insecticides are just as effective, and perhaps more effective, than emulsion sprays for control of the European corn borer.

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Insects in Stored Peanuts and Their Seasonal Abundance[1]

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Many insects which infest stored grain, milled products, dried fruits, and other foods also attack peanuts. The exact number of insect species infesting stored peanuts is not known. According to Popenoe (1911), the Indian-meal moth, *Plodia interpunctella* (Hbn.), is the most important insect pest of stored peanuts in the United States. Popenoe also listed other species of insects in order of their importance as follows: red flour beetle, *Tribolium castaneum* (Hbst.), saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), cadelle, *Tenebroides mauritanicus* (L.), almond moth, *Ephestia cautella* (Wlkr.), and the Mediterranean flour moth, *Ephestia kühniella* Zell. In reporting on the insect pests of shelled peanuts, Bissell & DuPree (1946) found that the saw-toothed grain beetle was the most abundant species. Chittenden (1911) listed peanuts as a food material of the larvae of the almond moth.

Research was initiated in Alabama in 1952 on insect pests of stored farmer-stock peanuts.[2] A list of the species of insects and their seasonal abundance are herein reported.

PROCEDURE.—In 1952, a total of 117 tons of farmer-stock peanuts was stored in 19 storage bins. The experiment was enlarged in 1953 to include 30 storage bins filled with 180 tons of peanuts. In general, the peanuts were placed in storage during October-November and emptied from the bins in August. In 1952, peanuts were placed in various types of storage bins at kernel moistures ranging from 5.3 to 13.4 per cent. In 1953, the range in moisture was from 6.9 to 14.0 per cent. In both years shortly after the peanuts were placed in storage, they came to an equilibrium moisture level of approximately 6 per cent. During the storage period, the moisture content fluctuated within a range of about 1 per cent from this level.

Insect infestations of the peanuts were determined at various intervals throughout the year. For the infestation counts, 500-gram samples of peanuts were removed from the bottom, middle, top sections and the surface of each bin, and the number and kind of insects occurring in the sample were recorded. Samples of peanuts were drawn with a special probe inserted through openings in the inner door of each bin. The number and kind of insects per 500 grams of peanuts were converted to the number and kind per bushel (22 pounds) of peanuts when the seasonal abundance by dates was computed.

In recording the number and kind of insects found in each 500-gram sample of peanuts, all coleopterous larvae were recorded under the heading of beetle larvae, and all lepidopterous larvae were recorded under the heading of meal moth larvae. In August, 1954, specific determinations were made on all larvae collected from the foreign material of each 500-gram sample of peanuts, and separate determinations were made on the larvae collected from the peanut kernels of a 100-gram sample. This study was made to determine what species of insects cause damage to peanut kernels and what species occur only in the foreign material and cause no apparent damage.

RESULTS.—Twenty-nine species of insects have been found in stored peanuts. Twenty-six of these species have been identified as follows:

Ahasverus advena (Waltl.)
Apanteles diacrisiae Gahan
Araecerus fasciculatus (Deg.)
Bracon hebetor Say
Carpophilus dimidiatus (F.)
Cathartus quadricollis (Guer.)
Cephalonomia tarsalis (Ashm.)
Dermestes caninus (Germ.)
Ectopsocus pumilis (Banks)
Ephestia cautella (Wlkr.)
Laemophloeus pusillus (Schonh.)
Lasioderma serricorne (F.)
Lyctocoris campestris (F.)
Mesostenus gracilis Cresson
Oryzaephilus surinamensis (L.)
Plodia interpunctella (Hbn.)
Rahbdepyris zeae Waterston
Rhizopertha dominica (F)
Sitophilus oryza (L.)
Sitotroga cerealella (Oliv.)
Tenebrio molitor (L.)
Tenebroides mauritanicus (L.)
Tribolium castaneum (Hbst.)
Tribolium confusum Duv.
Trogoderma sternalis Jayne
Typhaea fulmata L.

[1] Accepted for publication July 20, 1955.

[2] This study is being carried out as part of a cooperative project between the Commodity Credit Corporation, the Agricultural Marketing Service, U.S.D.A., and the Agricultural Experiment Station of the Alabama Polytechnic Institute.

Table 1.—Seasonal abundance of insects[a] in stored peanuts, Headland, Alabama, 1953–54.

Insect	Average Number Insects per Bushel of Peanuts					
	November	March	April	May	July	August
Beetle larvae[b]	31.8	1.2	2.3	5.0	18.7	18.4
Cadelle	0	0	0	0	0	1.8
Cigarette beetle	0	0	0.9	0	0	6.1
Coffee bean weevil	0.1	0	0.4	0	0	0.4
Corn sap beetle	26.9	4.7	16.8	42.5	736.2	398.6
Flat grain beetle	1.0	0	0	0	0	8.9
Flour beetles[c]	187.1	125.9	73.0	45.0	187.5	1103.7
Foreign grain beetle	134.4	57.6	45.2	20.0	41.3	50.4
Hairy fungus beetle	33.0	7.6	4.5	11.2	6.2	1.3
Hymenoptera[b]	42.5	12.9	10.2	3.7	7.5	5.4
Lesser grain borer	0	0	0	0	0	0.4
Lyctocoris campestris	4.1	3.5	18.0	22.5	266.2	132.3
Meal moth larvae[b]	13.9	3.5	1.3	1.3	8.7	2.1
Meal moths[b]	5.0	1.8	3.0	3.7	7.5	2.3
Trogoderma sternalis	0	0	0	0	0	0.9
Saw-toothed grain beetle	1.0	0	1.3	1.3	1.3	34.6
Square-necked grain beetle	3.8	0	0.7	0	25.0	0.7

[a] Refers to adult insects unless otherwise indicated.
[b] Includes several species.
[c] Includes red and confused flour beetles.

Six of these species were beneficial hymenopterous parasites. Three other species of insects have been found in the peanuts, but their specific identity has not been determined.

The seasonal abundance of the various species of insects is shown in table 1. The insect infestation of the peanuts in November, 1953, was quite high due to the presence of insects in the storage bins from 1952-crop. In general, the number of insects per bushel of peanuts was lower in March, April, or May, 1954, than in November, 1953. The greatest increase in most species of insects occurred during the summer months of July and August.

The most common beetles found in the peanuts in decreasing order of their abundance were: flour beetles, corn sap beetle, and foreign grain beetle. Flour beetles were quite abundant throughout the year and reached their peak infestation in August. The corn sap beetle was not numerous (4.7/bu.) during March, but by July its numbers increased considerably (736.2/bu.). The population of the foreign grain beetle was somewhat consistent throughout the year, its abundance peak being in November 1953.

The saw-toothed grain beetle was not found in large numbers until August. The hairy fungus beetle and hymenopterous parasites were more common in November than at any other time during the year. Considerable numbers of a true bug, *Lyctocoris campestris* (Fab.), were present in the peanuts, particularly during July and August. The importance of this species is not known.

The number of meal moths reported in table 1 does not reflect their true abundance. Active flight of meal moths prevents taking a true measure of their infestation by counting the number found in peanut samples. From observation, the Indian-meal moth was the most abundant species, followed by the almond moth.

Many of the pests of stored peanuts, such as square-necked grain beetle, cigarette beetle, coffee bean weevil, cadelle, flat grain beetle, lesser grain borer, and dermestid beetles, were not found in large numbers until August.

The seasonal abundance of insects in stored peanuts for the 1952–53 storage period is not included in this report. Because of the newness of the storage site, insects were not found in large numbers in the peanuts until July and August, 1953. The rice weevil, yellow mealworm, and a dermestid beetle, *Dermestes caninus* Germ., were found in 1952–53 stored peanuts but were not found in the 1953–54 stored peanuts.

Table 2.—Number and kind of larvae collected from the foreign material in stored peanuts and from peanut kernels, Headland, Alabama, August, 1954.

Insect Larvae	Number of Insect Larvae[a]	
	Peanut Kernels	Foreign Material
Cadelle	4	7
Cigarette beetle	1	0
Corn sap beetle	12	23
Flour beetles[b]	57	73
Indian-meal moth	21	8
Saw-toothed grain beetle	5	0
Trogoderma sternalis	4	0
Unidentified	6	3

[a] Four 500-gram samples of peanuts taken from each of 30 storage bins and inspected for larvae in the foreign material; 100 grams of peanuts shelled in each sample and inspected for larvae in kernels.
[b] Includes larvae of red and confused flour beetles.

The number and kind of larvae collected from the foreign material and from peanut kernels are presented in table 2. More flour beetle larvae were found in the kernels and in the foreign material than any other species of insect. Indian-meal moth larvae and corn sap beetle larvae were the next most abundant species, respectively. Almost three times as many Indian-meal moth larvae were collected from peanut kernels as were collected from foreign material. Conversely, almost twice as many corn sap beetles were found in the foreign material as were found in peanut kernels. Larvae of the saw-toothed grain beetle, dermestid beetles, and the cigarette beetle were collected from peanut kernels but were not collected from the foreign material.

SUMMARY.—Experiments were conducted in Alabama during 1952–54 on the insect pests of stored farmer-stock peanuts. Twenty-six species of insects were found infesting stored peanuts; of this number, six species were beneficial hymenopterous parasites.

The most common stored grain beetles found in the peanuts in order of decreasing abundance were: flour beetles, *Tribolium castaneum* (Hbst.) and *T. confusum* Duv., corn sap beetle, *Carpophilus dimidiatus* (F.), and foreign grain beetle, *Ahasverus advena* (Waltl.). The Indian-meal moth, *Plodia interpunctella* (Hbn.), was the most abundant moth. The greatest increase in number of insect pests occurred during July and August. Hymenopterous parasites were more abundant in November than at any other time during the year. Studies were made of the larvae of insect species infesting peanut kernels. This study revealed that, in order of decreasing abundance, the more numerous species were: flour beetles, Indian-meal moth, and corn sap beetle.

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Biology of the Black Flies in Jasper County, South Carolina, and Some Relationships to a *Leucocytozoon* Disease of Turkeys[1,2]

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Commercial turkey growers in South Carolina suffer considerable economic losses each year due to a disease in turkeys caused by a protozoon parasite, *Leucocytozoon smithi* (Lavern & Lucet), and commonly called gnat fever or turkey malaria. The ability of a black fly, *Simulium occidentale* Townsend, to transmit this disease to turkeys in Nebraska was shown by Skidmore (1932). O'Roke (1934) described a similar disease of ducks caused by *L. simondi* Mathis & Leger (=*anatis* Wickware), which is transmitted in Michigan by *S. venustum* Say. Twinn (1933) reported that a *Leucocytozoon* disease in ducks transmitted by this fly is common in eastern Canada. Two species of black flies, *S. jenningsi* Mall. (=*nigroparvum* Twinn) and *slossonae* D. & S., were reported by Underhill (1939) to feed on turkeys in Virginia, and *jenningsi* was demonstrated by Johnson *et al.* (1938) to transmit *smithi* to turkeys.

In Jasper County, one of several counties in South Carolina where the disease is prevalent, about 75,000 turkeys are produced each year. Annual death losses attributed to the *Leucocytozoon* disease in this one county average 5 per cent, or 3,750 birds. These birds have an average value of $3 each and represent a loss of $11,250. Blood smears from large flocks examined during this study have shown that all the birds become infected. No estimate has been made of the losses due to the disease in the 95 per cent that tolerate the infection.

According to the Agricultural Research Service (1954), the average annual production of turkeys in the United States during 1942–51 was 51,696,000 birds, valued at $239,668,000. The annual loss in mortality due to the *Leucocytozoon* disease during this period was $708,000, or 0.24 per cent of the production.

A study to determine the species of black flies[3] present in Jasper County, their biology, and their relationships to the *Leucocytozoon* disease of turkeys was conducted from February through the first half of June 1954.

DESCRIPTION OF TERRAIN AND STREAMS.—Jasper County is in the southern tip of the State, bounded on the south and west by the Savannah River, on the northeast by the Coosawhatchie River, and on the east by Beaufort County. Approximately half the area is swamp or marsh. The three main drainage systems are the Savannah River, the Coosawhatchie River and numerous small tributaries which form Broad River on the east, and New River, which has its origin in the north-central part of the county in Great Swamp and flows into the Savannah River at the southern tip of the county. Practically all the streams east of U. S. Highway 17 contain brackish water, at least during high tides, and do not harbor black flies. The streams west of this highway originate within the county and flow through heavily wooded and relatively flat terrain. Water velocity was found to vary from no visible water movement to 2½ feet per second. Riffles formed by logs, roots, accumulations of brush and leaves, and a few slight changes in elevation afforded practically all the black fly breeding areas. The streams have sand or mud bottoms and contain two or three types of submersed grass in open or partially shaded spots.

Larval Environments.—Water temperatures in the streams where immature black flies were found ranged from 42° to 55° F. when the intial survey was made in February, and averaged 75° on June 9. There was considerable flucutation during March and April as the air temperature varied. An 8° drop (from 59° to 51°) in water temperature was recorded in 5 days during early March and a 10° rise (from 56° to 66°) in 4 days during the latter part of the month. No adverse effects on the black flies were noted. The water temperature where *Simulium decorum* Walk. was found at lake outlets was slightly higher than at the streams, but this factor apparently had little effect on this choice of habitat.

The rate of water flow varied tremendously during this study, as would be expected in any area where drain-off is slow and the rainfall is infrequent or sporadic. Black fly breeding was stopped completely in several streams during May owing to lack of water flow but was resumed following rains. Subnormal rainfall during June and July eliminated breeding in all but 4 of the 12 streams in July, and by the middle of August breeding was found in only one of them. Some fluctuation in water level, and consequently in the density of larval and pupal populations, appears to occur every year in this area.

Water velocities at points where immature black flies were found ranged from about 12 to 24 inches per second. Although larvae were collected in flows as sluggish as 3.3 inches per second, they were not generally found where the normal velocity was below 10 or 12 inches. Very few rapids with speeds of over 2 feet per second were found. Water velocity within the limits of 12 to 24 inches per second did not appear to be a factor in the distribution of any particular species in the area.

FEEDING OF ADULTS.—Observations on adult black fly feeding were confined largely to turkeys; however, a few collections were made from cattle, and sweepings were made about the body of the senior author. *Simulium slossonae* and *congareenarum* (D. & S.) were collected from turkeys, *jenningsi* or related undescribed species from cattle, and all the species in Jasper County from humans. The senior author did not receive a single black fly bite, nor were they annoying, although they would land and crawl about on his clothing and exposed skin.

LIFE CYCLES AND GENERATIONS.—All the species except possibly *Cnephia pecuarum* (Riley) encountered in Jasper County have life cycles of approximately 3 to 4 weeks and have two or more generations each year. Breeding ceases when streams quit flowing and resumes following rains. Apparently the eggs withstand desiccation.

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[2] This study was made through the cooperation of the Entomology Research Branch, the South Carolina Agricultural Experiment Station, and the turkey department of the Cypress Woods Corporation.

[3] Alan Stone of the Entomology Research Branch, assisted in the initial survey and identifications of the black flies (Diptera: Simuliidae).

Simulium slossonae.—Larvae, pupae, and adults were taken from only two locations in Jasper County the first week in February. When the same streams were surveyed 1 month later, this species was found at seven locations, and by the latter part of March it was present in practically every flowing stream. Larvae and pupae were collected every week until June 11 and then monthly through August. On May 10 no black flies could be found at a few of the collection stations where the water had stopped flowing; one station was completely dry. A heavy rain (approximately 2.5 inches) fell on May 12. The next visit to these stations on May 20, revealed a high population of larvae, and 3 weeks after the rain pupae were abundant. This was considered good evidence that the life cycle was less than 1 month.

Most of the larvae were found in partly shaded segments of streams at road crossings and were attached to tape grass, which has long trailing leaves about $\frac{3}{4}$ inch wide. The highest concentration found consisted of 26 larvae per square inch of leaf surface, and the largest breeding area was about 100 square yards. This count was made 14 days after the rain on May 12. Not more than half this population survived to emerge, owing to the drying of the stream.

Similium congareenarum.—This was the most abundant and widespread species collected early in February. The flies were breeding in all the larger streams as well as most of the smaller ones. By mid-March the numerous small streams, which are strictly wet-weather streams, were drying up, and breeding was stopped before *slossonae* became established in them. After these small streams were dry, the two species were generally found breeding in the same locations. However, by the first of May *congareenarum* began decreasing in abundance while *slossonae* was increasing. An occasional larva and pupa were collected through June 11 and one larva was taken on July 13, but no adults were seen after May 15. The larvae attached mainly to aquatic vegetation in association with *slossonae* and the *venustum* group.

Simulium decorum.—This species was taken at three locations in Jasper County. Two of these sites were concrete spillways at artificial lakes, and the third, which had the smallest population, was at a floodgate-type outlet to a 60-acre duck pond. One spillway had a vertical wall about 5 feet high and 25 feet wide. The other was slightly wider and had an apron about 30 feet long with a slope of about 4 inches per foot. Although larvae attached to the sloping surface, they were concentrated on the dam with the vertical surface, where a thin film of water trickled over them. On April 5 a count of 442 larvae per square inch was made. Most of these larvae died before emerging owing to insufficient flow of water. Overlapping generations from early February until early May suggest that in years of normal rainfall this species continues to breed throughout the summer with a generation about every month.

Simulium venustum Complex.—*S. venustum* Say, *tuberosum* (Lundstr.), and at least one undescribed species identified from streams in the area are referred to in this report as *venustum* complex since separation in the field is impossible. No evidence could be found that they were involved in the transmission of the *Leucocytozoon* disease; hence, specific identifications of the collections have not been made. A thorough study may reveal a succession of species, as suggested by Dr. Stone. They were collected weekly during the study and were widespread but never abundant. Although in general they were breeding in association with *slossonae* and *congareenarum*, they had a tendency to extend into densely shaded areas and attach more to dead leaves and brush. Life cycles were about the same as for *slossonae* and *congareenarum*.

Cnephia pecuarum.—Adults were observed on the wing the first week in February as well as from March 1 to 15. Since larvae and pupae were taken from only seven locations in the county and in relatively small numbers, no specific data were obtained to indicate more than one generation. All seven collection points were on the drainage side of ponds and boggy areas, where stagnant water was present. The larvae and pupae were attached to dead and green vegetation in partially shaded areas.

SOME RELATIONSHIPS OF BLACK FLIES TO LEUCOCYTOZOON DISEASE.—Observations on the feeding of black flies on turkeys were made by taking young turkeys, 6–10 weeks of age, away from the main flock into areas where the flies were breeding. A few *congareenarum* and *slossonae* were attracted to turkeys in about equal numbers during the first half of May. However, adult activity of *congareenarum* decreased rapidly to zero by the middle of the month and the abundance of *slossonae* increased. On the last day of observations, June 11, on an average 10 flies per minute were feeding on an isolated turkey. Adults of both species usually fed on the head and neck and occasionally on the wing or body where the skin was exposed owing to loss of feathers. They usually lit directly on these exposed areas, and did very little crawling before biting. Most of them engorged in a minute or two. Immediately after engorging, the flies flew away so rapidly that their flight could not be followed. They were observed feeding in a wide range of weather conditions, such as temperatures from 66° to 102.5° F., dark cloudy (even light rain) to bright sun, presence or absence of slight breeze, and throughout the day from early morning until dusk. No night feeding observations were made; however, an occasional *slossonae* was taken from a light trap near the turkeys.

A young bronze turkey harboring many *Leucocytozoon* parasites in its blood stream was used as a host in collecting engorged flies for disease-transmission studies. Females of *slossonae* were collected with an aspirator from this bird and transferred to a pint ice-cream carton covered with two layers of cheesecloth. The carton of flies was kept in a dark, cool place with a moistened cotton plug on the cloth while being carried by the junior author to the laboratory at Clemson College. Most of the flies were dead by the time they arrived. Ten of the dead flies were macerated in 1 ml. of physiological saline by grinding between two glass bottle stoppers. Three more milliliters of saline were added, and 1 ml. of this inoculum was injected intravenously into each of four disease-free 10-week-old turkeys. One bird was sacrificed for tissue-section studies 3, 6, 10, and 13 days after inoculation. The bird sacrificed after 13 days showed many gametocytes in smears from the blood and lungs. One fly was still alive 5 days after feeding on an infected bird. It was transferred to a gelatin capsule and permitted to feed on a disease-free 10-week-old turkey. Sixteen days later gametocytes were found

in smears of the peripheral blood. Adults of *congareenarum* were not available for this study.

Turkeys reared under screened conditions in a disease-free area were brought into the troubled area to determine how soon they would become infected. Blood smears taken daily from several birds showed that gametocytes of the *Leucocytozoon* parasites appeared in the blood stream in about 15 days after being exposed to black flies. This interval confirms the grower's statement that the birds begin dying 2 to 3 weeks after they are moved from the brooder house to the range.

Blood smears taken from turkeys at the time they were moved from the brooders at 8 weeks of age showed that some were infected. The rate of infection in this flock of 2,000 birds sampled at random is shown below:

Age of birds (weeks)	*Number positive*	*Number negative*
8	1	75
10	10	20
11	24	6
12	26	4
13	30	0

SUMMARY.—Economic losses in the commercial production of turkeys in South Carolina due to a *Leucocytozoon* disease transmitted by one or more species of black flies (*Simulium*) prompted biological studies of the flies and their relationships to the disease in Jasper County during 1954. *Simulium slossonae* D. & S., *congareenarum* (D. & S.), *decorum* Walk., *venustum* Say, *tuberosum* (Lundstr.), *jenningsi* Mall. (or a related species), *Cnephia pecuarum* (Riley), and an undescribed species of *Simulium* were collected in the county. Only *S. slossonae* and *congareenarum* were found feeding on turkeys. *S. slossonae* was shown to be a vector of the disease.

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Greenhouse Tests on Control of the Beet Leafhopper[1]

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Southern Idaho produces approximately 80 per cent of the national requirement of snap bean seed, valued at about $83½ million annually. The beet leafhopper, *Circulifer tenellus* (Bak.), limits the area where these beans can be successfully grown. This insect does very little feeding on beans, but less than a minute of feeding by a viruliferous leafhopper is required to transmit the virus of curly-top disease to the young plants.

In 1946 a project was started at Twin Falls, Idaho, to find a direct method of controlling the beet leafhopper on snap beans. Since insecticide injury to beans often occurred, laboratory tests were also designed to explain this damage. Results of the field tests under this project have been reported (Deen & Hallock 1954). The data from greenhouse tests on 33 insecticides between 1947 and 1952 are presented in this paper. The materials were evaluated on (1) their toxicity to the beet leafhopper, (2) their protection of snap bean plants from curly-top infection, and (3) their phytotoxicity to snap bean plants.

TOXICITY.—In the toxicity tests the sprays were applied to individual potted plants as they were revolved on a turntable. The sprays were discharged from a compressed-air sprayer at 40 pounds' pressure. Sugar-beet plants were used, as the beet leafhopper usually does not survive long on bean plants. In 1950, however, a few tests were made on Bountiful beans, a variety that is very susceptible to curly top.

Single-leaf cages were used on sugar beets and a cage over the entire plant on beans (Fig. 1). Ten or five leafhoppers were placed in each cage on beets and beans, and

FIG. 1.—Left, three leaf cages on a sugar-beet plant; right, cage over entire bean plant.

the treatments, including a check, were replicated from 6 to 126 times.

The control 4 days after exposure as calculated from the leafhopper mortalities on treated and untreated plants is shown in tables 1 and 2. Except where noted, all spray formulations were emulsions, and were applied at 100 gallons per acre. Corn oil was used as a solvent, and the

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[2] This work was conducted under the supervision of J. R. Douglass. E. W. Jones and V. E. Romney also assisted in these tests.

Table 1.—Results of tests with various insecticides for control of the beet leafhopper on sugar-beet foliage.

INSECTICIDE	STRENGTH	REPLICATES	CONTROL
	Per Cent	*Number*	*Per Cent*
Dusts:			
Allethrin	0.5	6	71
Pyrethrum	.4	6	100
Dilan	5	10	90
DDT, purified	5	26	96
DDT, technical	5	28	96
Ditolyl trichloroethane	5	6	84
Methoxychlor	5	12	98
Perthane	5	16	100
	Pounds per 100 gallons		
Sprays:[a]			
Aldrin	1	6	1
	1.5	40	20
Aramite, WP	1	74	14
Pyrolan	1	18	36
Colorado 9	1.5	60	67
Prolan	1	16	56
Bulan	1	16	52
DDT, technical	1	58	85
	1.5	20	72
DDT, purified	1	126	93
	1.5	40	86
	2	20	100
DDT, analog A, WP	1	10	65
DFDT, WP	1	32	84
Dieldrin	0.6	6	3
EPN	1.5	22	100
Heptachlor, WP	1	22	45
Lindane	4	10	100
Parathion	1	20	100
Perthane	1	28	84
Tert.-Butylvalone	1	10	8
TDE	1	10	78
Toxaphene	1.7	20	23
+piperonyl butoxide	1.7 of each	20	53
Valone	1	10	9
	Per Cent		
Schradan	0.17	60	80
	.1	60	52
	.05	20	73

[a] Emulsions except where WP is indicated.

Table 2.—Results of tests with various insecticides for control of the beet leafhopper on Bountiful beans.

INSECTICIDE	STRENGTH	REPLICATES	LEAFHOPPER CONTROL	CURLY-TOP REDUCTION
	Per Cent	*Number*	*Per Cent*	*Per Cent*
Dusts:				
Prolan	5	30	95	69
Bulan	5	30	100	80
Ditolyl trichloroethane	5	30	100	51
	10	15	100	40
DDT, purified	5	15	100	50
Methoxychlor	5	30	99	56
	10	15	100	49
	Pounds per 100 gallons			
Sprays:				
Prolan	1.5	15	85	17
Bulan	1.5	15	99	17
DDT	1.5	90	94	29
TDE	1.5	15	81	80
	Per Cent			
Schradan	0.125	15	99	17
	.25	15	79	26
	.5	15	69	14
	.75	15	66	34
	1	15	51	74
Drench:				
Schradan	1	15	100	100
	.75	15	100	20

emulsifier contained 80 per cent of polyalkylaryl polyether alcohol and 20 per cent of isopropanol. Three analogs of DDT—analog A (1,1,1-trichloro-2-(*p*-chlorophenyl)2-phenylethane), Colorado 9 (1,1,1-trichloro-2,2-bis-(*p*-bromophenyl)ethane), and DFDT (1,1,1-trichloro-2,2-bis(*p*-fluorophenyl)ethane)—showed from 65 to 84% control. The phosphorous compounds EPN and parathion gave 100% control. Nine materials gave over 90% control. The addition of equal parts of piperonyl butoxide to toxaphene gave 53% control of leafhoppers, as compared with 23% for toxaphene alone.

PROTECTION FROM CURLY TOP.—From 1947 to 1951 tests were made on the value of insecticide treatments in the protection of nonresistant sugar beets and beans from curly-top infection. The sprays were applied as in the toxicity tests, and the dusts were applied with a hand duster as the plants revolved on a turntable. Each plant was grown in a 4-inch pot. After treatment, 36 plants representing 6 to 12 replicates of 3 to 6 treatments were placed at random in a 30×30×14 inch cage. The plants in each cage were exposed to 4 groups of 50 viruliferous leafhoppers for 24-hour periods. All plants and surviving leafhoppers were removed from the cages in 10 days, and the final reading of curly-top symptoms was made after 30 days.

The curly-top reduction ranged from 0 to 96 per cent on sugar beets (Table 3) and from 14 to 100 per cent on Bountiful beans (Table 2). Although schradan gave high leafhopper mortality, only concentrations of approximately 1 per cent showed good curly-top reduction on beans. Plants treated with emulsions, dusts, or mists of

Table 3.—Results of tests with various insecticides for control of the beet leafhopper on sugar-beet foliage.

INSECTICIDE	STRENGTH	REPLICATES	CURLY-TOP REDUCTION
	Per Cent	*Number*	*Per Cent*
Dusts:			
DDT, purified	5	8	49
	10	17	80
Methoxychlor	5	8	41
Bulan	5	8	49
Prolan	5	8	64
Toxaphene	10	9	18
Pyrethrins+piperonyl cyclonene	4+5	7	32
Zinc nicotinyl fluosilicate	1	7	4
Chlordane	10	9	27
	Pounds per 100 gallons		
Sprays:			
DDT, technical	4.5[a]	9	96
	1	38	50
	1.5	28	64
DDT+Perthane	0.5 of each	4	0
Colorado 9	2	10	82
Toxaphene	3	9	3
Piperonyl cyclonene	2.6	9	6
BHC	5	7	1
Chlordane	2	9	31
Prolan	1	8	27
Bulan	1	8	23

[a] Applied as a mist in 7 gallons.

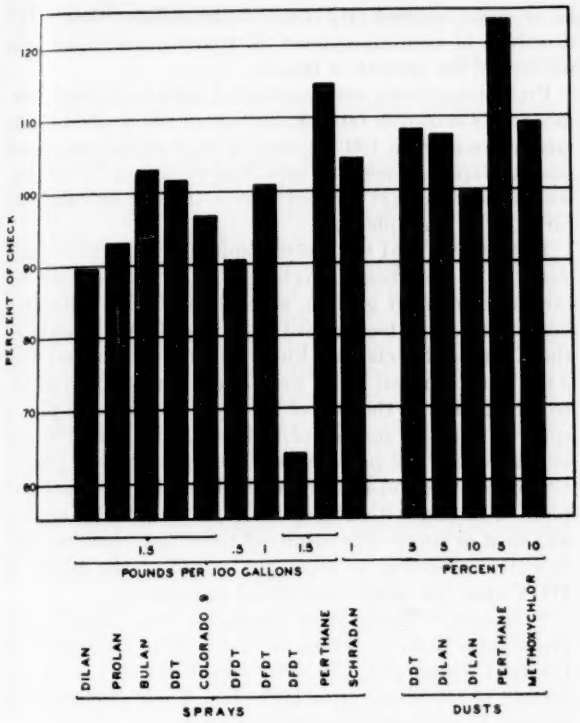

FIG. 2.—Effects of insecticides upon height of Bountiful bean plants.

DDT and with emulsions of Colorado 9 and TDE showed more than 50% reduction of curly top. In some of the tests plants treated with Prolan, Bulan, and methoxychlor reduced curly top effectively.

Plant hormones applied as sprays, seed soaks, or soil drenches reduced curly top from 6 to 61 per cent, as shown in table 4. Naphthaleneacetic acid gave the most promising results and should be further tested. Curly top was also reduced by 2,4-D, but this material retarded the growth of the plants and caused them to be deformed.

Table 4.—Curly-top infection of Bountiful snap bean plants and plant height as affected by the application of plant hormones at 25 p.p.m. in a 1-hour seed soak, as a spray applied in the crookneck stage, and as a drench at 25 ml. per plant.

PLANT HORMONE	PER CENT CURLY-TOP REDUCTION			PLANT HEIGHT—RATIO TREATED TO CHECK (PER CENT)		
	Seed Soak	Spray	Drench	Seed Soak	Spray	Drench
2,4-Dichlorophenoxyacetic acid	7	28	50	86	91	99
β-Naphthoxyacetic acid	19	14	29	106	111	93
Indolebutyric acid	47	17	21	113	112	90
Naphthaleneacetic acid	61	42	48	115	130	108

PHYTOTOXICITY.—The effects of the insecticides upon the bean plants were determined 30 days after treatment by comparing the height of the treated and untreated plants that grew under the same conditions.

Figure 2 compares the growth of Bountiful bean plants treated with 15 insecticide formulations with untreated plants. Most of these sprays were emulsions, but a DDT wettable powder was used in a few tests. All materials were applied as described above at a strength of 1.5 pounds per 100 gallons, and in some tests DFDT and schradan were used at lower strengths. The untreated plants ranged from 8 to 21 inches in height, depending upon the temperature. DFDT at 1.5 pounds caused severe plant stunting, as shown in figures 2 and 3. When the strength was reduced to 0.5 pound, the height of the plants was 90 per cent of that of the untreated plants. Plant stunting did not occur in two tests with 28 replicates where 1 pound of DFDT was used. Schradan applied at 1% concentration as a drench caused no plant stunting, and table 2 shows good control and curly-top reduction in a few preliminary tests.

Perthane increased the plant height significantly both as a spray and as a 5% dust. Deen & Hallock (1954) recorded an increased yield where a dust containing 2.5 per cent each of Perthane and DDT was used in one field experiment, but additional data should be obtained to

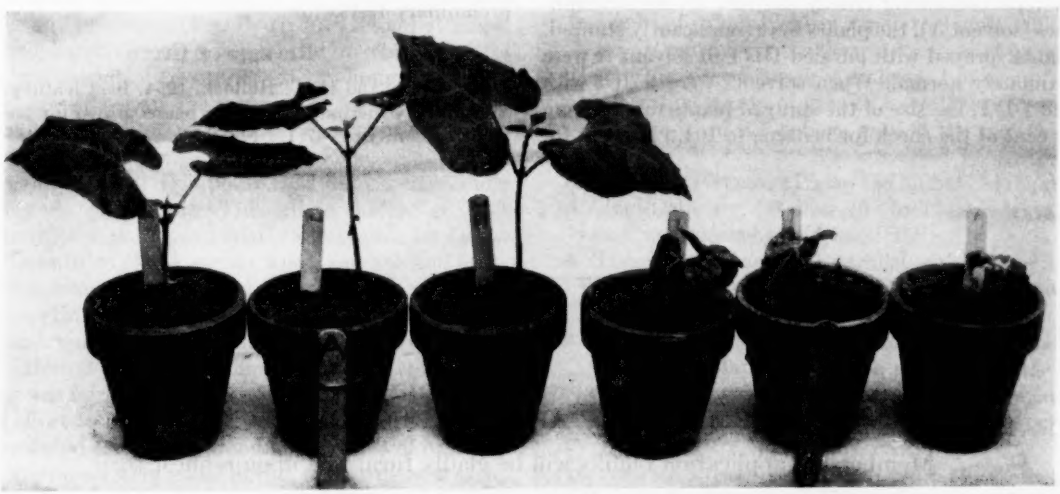

FIG. 3.—Bountiful bean plants dwarfed by insecticide: (A) Three untreated plants; (B) three plants sprayed with 1.5 pounds of DFDT per 100 gallons. Photographed 10 days after treatment.

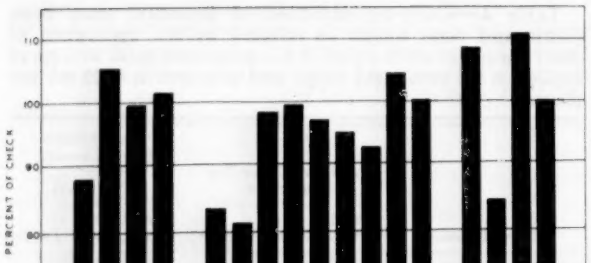

FIG. 4.—Height of Bountiful beans where DDT was used as dusts or as sprays in different solvents. S.A. in column 5 is solvent A.

evaluate this material. Dusts containing 5 per cent of DDT, 5 and 10 per cent of Dilan, or 10 per cent of methoxychlor were not injurious to the plant growth. Plants dusted with 10% Dilan were of normal size but not as large as those receiving the 5% Dilan. Beans sprayed with Dilan and Prolan averaged 90 per cent of the height of untreated beans.

Since DDT appeared to be one of the most promising insecticides, it was tested in 18 formulations, which are compared in figure 4. Dusts were generally safer than sprays. A 10% technical-DDT dust significantly reduced the height of the beans, but plants dusted with a 10% purified DDT dust were normal. Plants sprayed with technical DDT were generally smaller than those sprayed with purified DDT, which showed no significant reduction in size. Although other tests showed that plants sprayed with the different solvents without DDT were normal, there was a noticeable difference in the size of those sprayed with DDT dissolved in some of the solvents. When technical DDT was dissolved in a nonvolatile solvent containing chiefly di- and tri-methylnaphthalenes (solvent A), the plants were significantly stunted, but plants sprayed with purified DDT in solvent A were approximately normal. When solvents were tested with purified DDT, the size of the sprayed plants ranged from 93 per cent of the check for benzene to 104.6 per cent for an aromatic solvent (B). These tests indicate that DDT dissolved in corn oil, solvent B, toluene, and xylene do not retard the growth of beans.

Preliminary tests were conducted with four plant hormones. As shown in table 4, the size of the treated beans ranged from 86 to 130 per cent of that of the untreated plants. Naphthaleneacetic acid in applications by drench, seed soak, or sprays did not reduce the size or cause injury to the bean plants.

In 1950 and 1951 tests were conducted on 12 additional varieties of snap beans. Technical DDT caused some retardation of plant growth, as compared with untreated beans and those treated with purified DDT. Plants of the Kinghorn Special and Fullgreen varieties dusted with 5 per cent technical DDT were 72 per cent as high as untreated plants at the end of 30 days. Comparable plants sprayed with 1.5 pounds of technical DDT per 100 gallons averaged 79 per cent as high as untreated plants. Cherokee Wax and Dwarf Horticulture did not show any plant dwarfing with technical or purified DDT applied as a dust or spray. The results of these tests were not sufficiently consistent to allow evaluation of the effects of DDT upon the other varieties of snap beans.

SUMMARY.—Thirty-three chemicals were compared in greenhouse tests to determine their value for control of the beet leafhopper, *Circulifer tenellus* (Bak.), and protection of the plants from curly top. Their phytotoxicity to snap bean plants was also observed.

The chemicals were applied as dusts and sprays. The data were taken 4 days after treatment for the leafhopper control and after 30 days for protection from curly top and effects upon plant growth. Although several of the materials gave good leafhopper control, only Bulan, Colorado 9, DDT, schradan, and TDE showed as much as 80 per cent reduction in curly top. Purified DDT and Perthane were found to be safe on Bountiful beans when applied at a concentration of 1.5 pounds per 100 gallons. Emulsion sprays of technical DDT dissolved in a commercial preparation containing chiefly di- and tri-methylnaphthalenes caused significant stunting of snap beans. The plant hormone naphthaleneacetic acid at 25 p.p.m. was safe on Bountiful beans and decreased curly top in the preliminary tests.

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The Rearing of *Lyctus planicollis* and the Preparation of Wood for Control Tests[1]

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The southern lyctus beetle, *Lyctus planicollis* Lec., (Fig. A) causes extensive and costly damage to seasoned wood products. The wood species most commonly attacked are oak, hickory, ash, and walnut, all of which have characteristics making them highly desirable for commercial uses. This insect has been the subject of considerable research for a good many years, principally to determine salient life history facts and to develop effective and economical control measures. In recent research on the chemical control of this species a method was developed for rearing easily and quickly large numbers of beetles for use in tests. This paper describes the method and includes typical results of its application.

Briefly, attack on wood is initiated in this manner: a female rests on the surface of the wood and inserts her ovipositor into the pore opening of a springwood vessel. She then extends the ovipositor down the vessel, depositing eggs within the first 7 mm. from the pore opening. After hatching, larvae bore through the wood parallel with the grain, reducing it to a fine powder. Three to nine months later, when metamorphosis is complete, the new generation of adults bore out through the wood surface, making the typical small circular holes.

The need for an improved culturing procedure became obvious very early in the chemical control studies. The first beetles were obtained from 3500 infested implement handles. Though the larval population was high, natural enemies were very abundant. Over a 5-month period only 1000 beetles were collected, while 1000 predators (*Tarsostenus univitattus*) and 9000 parasites (*Monolexis lycti*) were gathered. In contrast to this, the procedure to be described produces about 5000 beetles per day and virtually no parasites.

In developing the method, several kinds of culturing material were investigated. The kind first used was infested implement handle stock. It was discarded when it was found impossible to eliminate the large numbers of parasites and predators (Fig. B, 1). In a second attempt, uninfested handle stock was exposed to the attack of beetles under controlled conditions to eliminate natural enemies. Nevertheless, the number of beetles produced still remained too low for large-scale testing purposes (Fig. B, 2).

It became clear at this point that implement handle stock was not satisfactory. This led to the use of fresh wood considered to be particularly susceptible to *Lyctus* attack. Quantities of oak sprout wood and ash and hickory sapling wood were collected during the dormant period of growth, when it contained a maximum amount of starch, and seasoned in the laboratory. This wood was quickly infested and the number of beetles ultimately emerging was fairly satisfactory (Fig. B, 3).

In an effort to further improve the method, limbwood was substituted for sapling wood. Collections of oak and hickory limbwood were made during the dormant season, processed, seasoned, and exposed to beetle attack. The results were decidedly superior to those obtained in previous studies, and this material is now used for the large-scale rearing of beetles for testing purposes (Fig. B, 5).

FIG. A.—Adult of *Lyctus planicollis* X-15).

Having determined the most suitable breeding material, there remained the task of developing a procedure for its most effective use. The method finally devised is as follows:

1. Limbs of oak or hickory 1 inch to 3 inches in diameter are cut during the dormant season of growth before the stored starch is utilized. Limbs cut during the active season of growth have little or no starch since most of it has been used in the growth of the tree.[2]
2. The bark is removed from the limbs.
3. The limbs are cut into 10- to 12-inch lengths for ease in processing and handling.
4. These lengths are split parallel with the grain, into pieces less than 1-inch thick, preferably between $\frac{1}{2}$ to $\frac{3}{4}$ inch, to facilitate seasoning.
5. The green wood is seasoned to below 18% moisture content. A standard electric moisture meter is accurate and rapid for determining moisture content. It is important that the wood be below 18 per cent;

[1] Accepted for publication July 29, 1955.

[2] The iodine test is employed for detecting starch. A concentrate is made by adding 2.4 gr. of iodine and 24 gr. of potassium iodide to 40 gr. of water. A working solution is prepared by diluting 1 part of the concentrate in 24 parts of water.

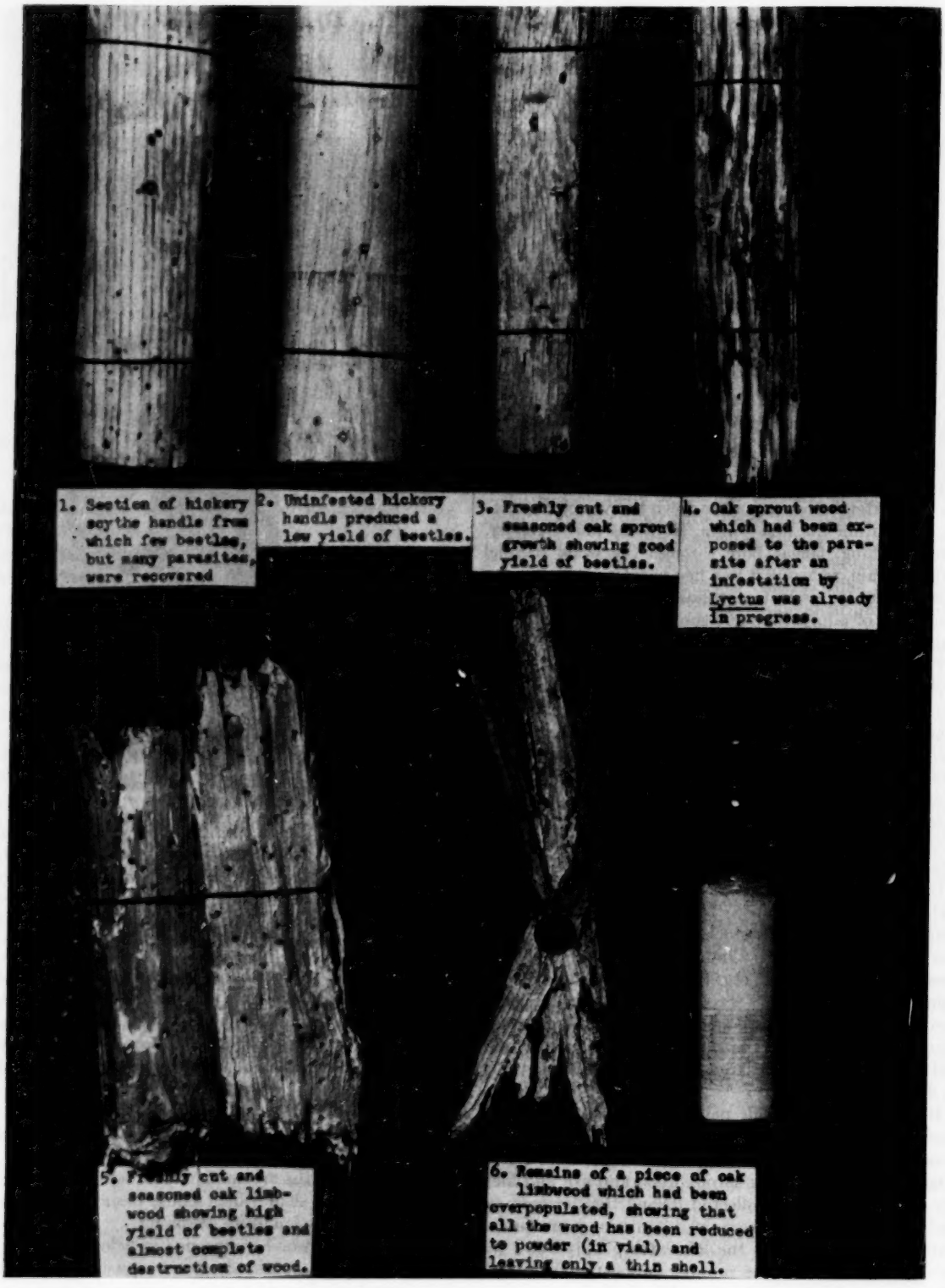
1. Section of hickory scythe handle from which few beetles, but many parasites, were recovered
2. Uninfested hickory handle produced a low yield of beetles.
3. Freshly cut and seasoned oak sprout growth showing good yield of beetles.
4. Oak sprout wood which had been exposed to the parasite after an infestation by Lyctus was already in progress.
5. Freshly cut and seasoned oak limbwood showing high yield of beetles and almost complete destruction of wood.
6. Remains of a piece of oak limbwood which had been overpopulated, showing that all the wood has been reduced to powder (in vial) and leaving only a thin shell.

FIG. B.—Samples showing various degrees of *Lyctus* infestation from different wood sources.

in fact the optimum moisture content is between 12 and 15 per cent. Though *Lyctus* will attack wood of greater moisture content, its breeding is handicapped by molds and mildews which develop when wood is confined in a container.

6. The seasoned wood is placed in a desired container.
7. Beetles are placed on the wood in the container and the open end of the container is covered with fine mesh muslin, chiefly to exclude natural enemies. The container is then placed in a warm room for

best results. There are many factors which determine the number of beetles to be added to wood for the best results. An adequate infestation will result from the attack of 10 to 12 beetles (the sexes are equally apportioned) on a piece of half-round limbwood 10 inches long and 1 inch thick. Of course, any multiple of this will be applicable. The exclusion of parasites cannot be emphasized too strongly.

8. When emergence of the new brood begins in the rearing containers and examination shows they are present in sufficient numbers for test purposes, a few sticks of freshly cut and seasoned wood are placed on top of the infested wood in the containers. Emerging beetles tend to congregate on this fresh wood (Fig. C). To collect beetles, these top pieces are removed and the beetles clinging to them are dislodged into an empty receptacle. Emergence of the new generation of beetles begins 3 to 9 months after the original liberation. If the culture wood is of high starch content, there will usually be a partial emergence of beetles 3 to 4 months after attack. Up to 30 per cent of the larvae may mature for this so-called short cycle. The remainder mature in the usual 9 to 12 months. The cause of this partial short cycle is not known but it could very likely be the result of certain conditions of moisture and starch or enzymes. This short cycle can be very useful in conducting more tests.

This method of preparing wood for breeding beetles as outlined above can, with certain modifications, be effectively used to produce wood in testing the effectiveness of insecticides for preventing beetle attack as well as for remedying existing attack. For remedial control tests, round sections of limbwood are sawed lengthwise. They are then seasoned by placing them in open stacks preferably in a heated greenhouse. Once they are seasoned they they are placed in closed containers with supplies of beetles. About 2 months after exposure to beetle attack the sections of wood are properly infested and ready for test purposes. Though there will be no emergence holes at this time, the degree of infestation of the wood can be easily estimated by cutting the 10-inch pieces in halves. The number of larval galleries visible on these exposed cross-sections is an accurate gauge of the number of larvae present in the wood.

For preventive control tests, the round pieces of wood are again sawed lengthwise. To season this wood, it is placed in cloth or fine screen-covered cages, in order to prevent it becoming infested by stray beetles which are usually present in the laboratory. A heater fan can be directed into this cage to hasten seasoning and to prevent the formation of mildew. After seasoning, the wood is stored in cloth-covered cans or cages until ready for use. The wood to be used in preventive control tests should be separated into small lots for all steps in the process, in order that it may be out of containers for as short a time as possible. Otherwise, stray beetles will infest the wood and render it unfit for preventive testing purposes.

FIG. C.—A can of *L. planicollis* culture. Several pieces of freshly cut uninfested wood have been placed on top of infested wood for collecting purposes.

SUMMARY.—In order to conduct extensive research on the control of *Lyctus planicollis* it was necessary to develop a culturing method which would produce an adequate supply of beetles with a relatively small amount of work. Earlier methods too often permitted loss of large numbers of beetles through the attack of parasites and predators or poor production through the use of low quality culture media. A method is described which overcomes both of these obstacles.

Oak or hickory limbs are cut during the dormant season of growth in order to obtain wood with a maximum of starch. The bark is removed from the limbs, which are then sectioned and split into convenient sizes to facilitate handling and seasoning. After being rapidly seasoned, the sections are exposed to beetle attack in closed containers. A small percentage of the resultant generation will mature in 3 to 4 months. However, the great majority will emerge in 9 to 12 months. Collecting the emerging beetles from the culture containers is greatly facilitated by placing freshly cut and seasoned limbwood on the top of the culture wood. The beetles are attracted to this fresh material and thus are easily and quickly recovered.

With slight modifications, the method used to prepare culturing wood can be very effectively utilized to produce a good homogeneous stock of material for conducting both remedial and preventive control tests with insecticides.

Transmission of Resistance to Chlordane in the German Cockroach[1]

J. M. Grayson, F. E. Jarvis, Jr., and M. Levitan, *Virginia Polytechnic Institute,*[2] *Blacksburg*

The literature relative to the genetics of resistance to insecticides has been reviewed by Cochran *et al.* (1953) and Grayson & Cochran (1955). Cochran *et al.* (1953) found that the inheritance mechanism for DDT resistance in the German cockroach is complex, with both chromosomal and extra-chromosomal factors being involved. King (1955), working with *Drosophila melanogaster,* has postulated that the character of resistance is multifactorial or polygenic in nature; his conclusion was based upon the selection of two lines of this insect species which apparently achieved resistance to DDT by consolidating different combinations of factors for resistance.

The results reported in this paper are those obtained from determining the relative toxicity of chlordane to: (a) chlordane-resistant and non-resistant (normal) strains of cockroaches, (b) the F_1 progeny from reciprocal crosses of these two strains, and (c) the progeny of back crosses. Further work on the toxicity of chlordane to the F_2 progeny of reciprocal crosses, the progeny of pair matings within strains, and the stability of resistance when selection is discontinued is in progress and will be reported later.

Procedure.—The cockroaches were reared in the same manner as previously described (Grayson 1951), except that cultures containing breeders and nymphal offspring were kept in a constant-temperature cabinet at 30° C. and 55 per cent relative humidity. Before the nymphs matured they were transferred to aquaria cultures and maintained at room temperature. Cockroaches within known age limits were obtained by removing all adults from the stock cultures at intervals of 7 to 10 days.

Technical grade chlordane was used in the toxicity tests. It was dissolved in acetone to form stock solutions, from which the desired amounts were volumetrically measured in preparing the water suspensions. To obtain satisfactory water suspension of chlordane, especially at higher concentrations, EMCOL H65A[3] was added in equal volume to the chlordane. Preliminary trials revealed that neither acetone nor the suspending agent exerted any deleterious effect upon the cockroaches at the concentrations employed.

For treatment purposes the males and females were placed separately in small screen cages, usually 25 to 30 per cage, and dipped in the insecticidal preparations at 30° C. They were held in the insecticidal preparation for 20 seconds and then allowed to remain in the treated cages for 60 minutes before removal to recovery jars. Adequate food and water were provided during the subsequent period of observation. Mortality counts were made at the end of 3, 6, and 10 days following treatment. Dead and moribund cockroaches were combined in the mortality counts. The criterion for regarding an individual as moribund was inability to exhibit active locomotion.

The tests at each concentration were usually replicated from 3 to 5 times, and at least four concentrations were employed in establishing the response of each strain. The data obtained were plotted on logarithmic-probability paper, and regression lines were fitted to the points by the method of least squares (Bliss 1935). Fiducial limits were determined for those lines occurring close together.

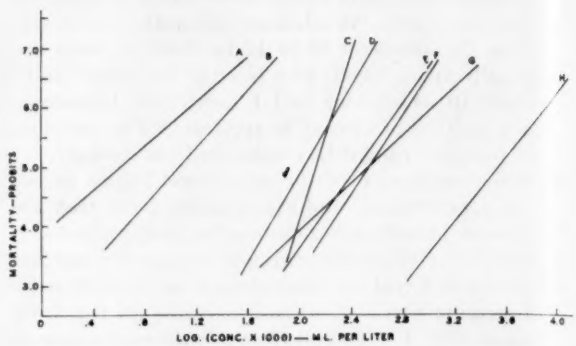

Fig. 1.—Mortality in the strains of the German cockroach involved in the reciprocal crosses after 10 days following treatment with chlordane. Non-resistant strain males, (A); non-resistant strain females, (B); Y-strain males, (C); X-strain males, (D); Y-strain females, (E); X-strain females, (F); chlordane-resistant strain males, (G); and chlordane-resistant strain females, (H). The X strain resulted from crossing resistant females with non-resistant males, and the Y strain from non-resistant females and resistant males.

Results.—The regression lines indicating the toxicity of chlordane to resistant and non-resistant strains, and to F_1 progeny arising from the reciprocal crosses of these two strains, are shown in figure 1. The orders of resistance at LD-50 and LD-90 of these strains, as well as those of selected back crosses, are presented in table 1. Males and females from the cross between chlordane-resistant females and non-resistant males are referred to as the X strain; and males and females from the cross between non-resistant females and chlordane-resistant males are designated as Y strain. The selected backcrosses involved mating F_1, Y-strain females with parent-type, non-resistant males; and F_1, Y-strain females with parent-type, resistant males.

Table 1.—Relative toxicity of chlordane to different strains and crosses of the German cockroach.

	Chlordane (Ml. per Liter)		Times Resistant	
	LD-50	LD-90	At LD-50	At LD-90
Non-resistant males	0.0041	0.0192	1.0	1.0
X-strain males (F_1)	0.111	0.247	27.1	12.9
Y-strain males (F_1)	0.133	0.210	32.4	10.9
Y-strain males (back cross)[a]	0.0435	0.325	10.6	16.9
Y-strain males (back cross)[b]	0.0980	0.425	23.9	22.1
Chlordane-resistant males	0.3450	1.500	84.1	78.1
Non-resistant females	0.0117	0.0400	1.0	1.0
X-strain females (F_1)	0.353	0.870	30.2	21.8
Y-strain females (F_1)	0.282	0.745	24.1	18.6
Y-strain females (back cross)[a]	0.200	0.490	17.1	12.3
Y-strain females (back cross)[b]	0.280	3.250	23.9	81.3
Chlordane-resistant females	3.550	10.070	303.4	251.8

[a] F_1, Y-strain females back crossed with parent-type, non-resistant males
[b] F_1, Y-strain females back crossed with parent-type, resistant males.

The regression lines for the progeny of the reciprocal crosses fall between those of the parents (Figure 1). The inherently greater insecticidal resistance of female cock-

[1] Accepted for publication August 15, 1955.
[2] Professor of entomology, graduate assistant in genetics, and associate professor of genetics, respectively.
[3] Manufactured by the Emulsol Corporation, Chicago, Illinois.

roaches, as compared to the males, probably accounts for the chlordane-resistant male regression line falling near those of the X- and Y-strain females. The chlordane-resistant male line also crosses that of the Y-strain males at probit 3.8 (12 per cent mortality); this is attributable to the greater slope of the former. Actually, the chlordane-resistant strain males are considerably more resistant at both LD-50 and LD-90 than either the X- or Y-strain males.

These data indicate autosomal transmission of the factors for resistance to chlordane in the German cockroach. Involvement of autosomal factors is clearly indicated by the greater resistance of the Y-strain males over that of the non-resistant males. Here the only source of resistance was the chlordane-resistant males, from whom it is assumed the F_1, Y-strain males received only a full set of autosomes. Furthermore, autosomal inheritance demands that the results of the reciprocal crosses be identical. The fiducial limits of the regression lines for the females of the X and Y strains overlap for their entire length; and those for the males cross between probits 5.2 and 6.6. This would indicate that the two populations of males and females are similar even though they may not be identical. Apparently little or no dominance is involved in the activity of the autosomal factors.

No evidence is indicated for the presence of cytoplasmic or sex-linked factors. If sex-linked factors had been present, the F_1, X-strain males would have been more resistant than F_1, Y-strain males. If cytoplasmic factors had been involved both males and females of the F_1, X-strain would have been more resistant than those of the F_1, Y-strain.

SUMMARY.—The genetics of chlordane resistance in the German cockroach, *Blattella germanica* (L.), was investigated by determining the order of resistance of: (a) the chlordane-resistant and non-resistant (normal) strains of roaches, (b) the progeny from reciprocal crosses, and (c) the progeny of selected back crosses.

The toxicological technique consisted of dipping adults from each strain in aqueous suspensions of chlordane at different concentrations and observing mortality up to 10 days. The data obtained are presented in the form of regression lines, and as order of resistant at LD-50 and LD-90.

The results indicate that the factors for chlordane resistance in the German cockroach are autosomal. Apparently little or no dominance is involved in the activity of the autosomal factors. There was no evidence to indicate the influence of any cytoplasmic or sex-linked factors in the transmission of chlordane resistance.

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Tests of Insecticides for Control of Cucumber Beetles in Ohio[1]

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In Ohio the striped cucumber beetle, *Acalymma vittata* (F.), and the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* Barb., are very destructive in their feeding on cucurbits, especially the seedling stages, and also as vectors of cucurbit wilt. During investigations at Columbus and Chillicothe from 1945 to 1952 many of the new insecticides were tested against these beetles. Some results of laboratory and field experiments with these materials are reported in this paper.

PREVIOUS WORK.—Alexander *et al.* (1945) reported that DDT gave effective control of the striped cucumber beetle in Massachusetts, and similar results were obtained in other States. Brooks & Anderson (1947) reported that dusts containing BHC (1 per cent gamma isomer) and DDT (1 per cent) were effective against both species on cucumbers and cantaloups in Virginia. Sun *et al.* (1948) found that DDT and chlordane were very toxic to adult spotted cucumber beetles in laboratory and greenhouse tests in New York State. Walton (1947) found that chlordane, BHC, and DDT were toxic to adult spotted cucumber beetles in laboratory tests and to both species in field tests in Oklahoma. Dustan *et al.* (1946) reported complete kills of striped cucumber beetles in laboratory tests of DDT and BHC in Canada.

Various investigators have shown that cucurbits are sensitive to chlordane, toxaphene, DDT, and BHC. Hervey & Schroeder (1946) found varietal differences in the susceptibility of cucumber and squash plants to injury from DDT. According to Crowell & Morrison (1950), moisture on cucumber and squash plants is conducive to injury by some of the chlorinated hydrocarbon insecticides.

Michelbacher *et al.* (1952) reported that aldrin, dieldrin, and heptachlor gave good control of cucumber beetles on melons in California but increased the mite population. Recently Michelbacher *et al.* (1953) discussed the control of the western spotted cucumber beetle, *Diabrotica undecimpunctata* Mann., and the western striped cucumber beetle, *Acalymma trivitatta* (Mann.), as pests of melons in northern California. They reported successful control with a 3 per cent DDT dust containing 3 per cent of Aramite or 7.5 per cent of Ovotran and with a 2 per cent parathion dust. Dieldrin, aldrin, and heptachlor were also effective, and TEPP and malathion dusts gave promising results.

LABORATORY TESTS.—In laboratory tests during October 1952 the beetles were confined in 4-inch petri dishes

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that had been dusted by a vacuum method similar to that described by Farrar *et al.* (1948). Ten adult beetles were put into each dish and supplied with a slice of gourd for food. The insects were held for 24 hours at about 75° F. in a cabinet that was well lighted for about 12 hours by fluorescent lights. Moribund insects were recorded as dead. Replicates were made on different days. All dusts were tested at the 1 per cent strength.

The results of five replicates of the tests against striped cucumber beetles were as follows:

	Per Cent Mortality
Parathion	100
EPN	94
Dieldrin	94
Malathion	81
NPD	80
Aldrin	69
Endrin	59
Isodrin	45
Untreated	0
L.S.D. at 5% level	31

In four replicates of the same dusts against spotted cucumber beetles, malathion killed 95 per cent and all the others 100 per cent. Either the spotted beetles were the more susceptible to these materials or they were more active in picking up the residues from the treated petri dishes.

In similar tests of some other materials on the striped cucumber beetles the results of eight replicates were as follows:

	Per Cent Mortality
Lindane	98
Systox	94
Heptachlor	82
Perthane	64
Chlordane	30
Toxaphene	22
Dilan	14
A-42 (Arsenomethane As, As-disulfide)	14
Untreated	0
L.S.D. at 5%	26

FIELD TESTS.—The field tests were made in small plots in commercial plantings, and the insecticides were applied with knapsack sprayers and dusters. Applications were made at approximately weekly intervals as long as the beetles were present in appreciable numbers. The strengths of the sprays and dusts were kept low to obtain better comparisons of the different materials. The effectiveness of an insecticide was usually judged by the number of surviving beetles found on or under the plants,

Test 1.—Results of field tests in central Ohio with various insecticide dusts against the striped cucumber beetle, 1948–52.

DUST	POUNDS PER ACRE—APPLICATION	NUMBER OF BEETLES PER SAMPLE
Experiment 1, 1948, 2 applications (5 square-foot samples)		
Parathion 0.5%	16	1.2
1%	12	2.2
Chlordane 1%	17	20.6
2%	18	5.4
TDE 1%	22	2.2
Methoxychlor 1%	15	3.4
Ditolyl trichloroethane 1%	20	8.0
DDT 0.5%	18	5.4
Toxaphene 2%	13	10.4
Untreated	—	20.4
L.S.D. at 5% level	—	8.9
Experiment 2, 1949, 1 application (5 square-foot samples)		
Methoxychlor 1%	18	0.4
Bulan 1%	14	0.0
Rotenone 0.75%	19	1.2
Lindane 1%	21	0.8
DDT 1%	22	2.8
Toxaphene 1%	12	2.6
TDE 1%	12	3.0
Prolan 1%	9	3.4
Parathion 1%	10	3.2
Chlordane 1%	9	2.6
Untreated	—	12.8
Outside of treated area	—	186.0
L.S.D. at 5% level	—	4.9
Experiment 3, 1950, 1 application (1 plant per sample)		
Bulan 2%	69	1.1
DDT 1%	41	2.0
Methoxychlor 2%	56	2.2
Chlordane 2%	56	2.5
Lindane 2%	44	2.6
TDE 2%	44	3.2
Parathion 1%	53	4.0
Untreated	—	11.4
L.S.D. at 5% level	—	1.7
Experiment 4, 1950, 2 applications (5 plants per sample)		
Parathion 1%	20	1.0
Dieldrin 1%	20	1.2
Lindane 1%	18	1.0
Aldrin 1%	19	1.5
DDT 1%	28	1.7
Methoxychlor 1%	18	1.7
Bulan	21	1.8
EPN 2%	15	2.5
Untreated	—	16.2
L.S.D. at 5% level	—	5.7
Experiment 5, 1951, 4 applications (3 plants per sample)		
Lindane 1%	16	0.9
Dieldrin 1%	18	1.5
Aldrin 1%	15	2.4
Endrin 1%	17	3.9
Malathion 1%	13	4.6
Metacide 1%	13	5.4
Velsicol 49-CS-56	13	5.6
Isodrin 1%	12	5.8
Perthane 1%	16	5.9
L.S.D. at 5%	—	1.5
Experiment 6, 1952, 1 application (4 plants per sample)		
Dilan 2%	35	0.2
Parathion 1%	38	0.8
Endrin 1%	42	0.8
NPD 2%	51	4.8
A-42 2%	49	5.8
Untreated	—	70.8
L.S.D. at 5%	—	9.1
Experiment 7, 1952, 2 applications (2 plants per sample)		
Dieldrin 2%	26	0.2
Heptachlor 2%	15	0.0
EPN 2%	19	0.0
Lindane 2%	19	0.4
Isodrin 1%	28	2.6
DDT 2%	22	0.4
Malathion 2%	17	0.0
Aldrin 2%	16	0.0
NPD 2%	16	0.8
Untreated	—	59.8
L.S.D. at 5% level	—	14.9
Experiment 8, 1952, 3 applications (4 plants per sample)		
EPN 2%	28	0.0
DDT 2%	20	0.4
Lindane 1%	23	0.4
Heptachlor 1%	18	0.8
Endrin 1%	20	2.2
Malathion 1%	22	5.8
NPD 2%	24	7.2
Untreated	—	27.6
L.S.D. at 5% level	—	8.6

since it was often difficult to obtain data on yield at the convenience of the growers.

In some of the earliest tests in which DDT was used, 1 and 2 per cent dusts gave good control of the striped cucumber beetles, and apparently were better than a mixture of cryolite and copper sulfate and other dusts containing nabam, ryania powder, and basic copper sulfate. DDT sprays injured the plants.

In some small-plot tests with four replications in 1947, cucumbers and acorn, butternut, and Hubbard squash were dusted with chlordane, toxaphene, and parathion. All the dusts were effective against the striped beetles, but toxaphene caused severe damage to the cucumbers and the acorn squash and some injury to the other squash. The average results for all the crops were as follows:

	Beetles per Plant
Chlordane 2%	0.7
Toxaphene 5%	0.4
Parathion 1%	0.6
Untreated	7.9
L.S.D. at 5% level	5.8

In 1948 a number of dusts were tested against both species on cucumbers in randomized 1-row plots 50 feet long with 4 replications (experiment 1). As is shown in table 1, parathion, methoxychlor, and TDE were the most effective materials tested against the striped cucumber beetle. Many dead spotted cucumber beetles were also found in the plots, but there were no significant differences in the effectiveness of the materials. The toxaphene dust severely injured the plants, and some injury was caused by a BHC dust that was used in a similar experiment in the same field.

In 1949 some of the dusts were tested on pumpkins that were under heavy attack by the striped cucumber beetles (experiment 2). The plots were 2 rows wide and 36 feet long and were in randomized blocks with 5 replications. As shown in table 1, the numbers of beetles were greatly reduced on the treated plots, and many dead beetles were found under the plants. The control was uniformly good, with no significant differences between the treatments. However, the effects of the treatments were partially obscured by movement of the beetles, since the number decreased markedly on the untreated check plots but remained high in the remainder of the field that was untreated.

In some tests for control of the striped cucumber beetle on cucumbers in 1950 (experiment 3, table 1), Bulan was significantly more effective than TDE or parathion, but most of the dusts were fairly effective. Results of other tests in 1950 (experiment 4) do not show any superiority of Bulan over parathion. All the other materials also appeared to be effective. It was noted that the materials gave good control of the spotted cucumber beetle. The yield was higher on the plots treated with aldrin, dieldrin, or DDT than on those treated with methoxychlor. Also aldrin and dieldrin gave better yields than EPN, parathion or methoxychlor. In experiment 3 the plots consisted of 2 hills and there were 20 replicates. In experiment 4 the plots were 2 rows wide and 36 feet long with 6 replicates.

Data on control of the striped cucumber beetle obtained on cucumbers in 1951 (experiment 5, table 1) show that lindane, dieldrin, and aldrin were more effective than endrin, isodrin, malathion, Metacide, Velsicol 49-CS-56,[3] or Perthane. Against the spotted cucumber beetle there was a tendency for lindane, dieldrin, and aldrin to be more effective than the other materials. The plots treated with aldrin, dieldrin, endrin, and lindane made the best yields. No cucumbers were produced on the check plots because the plants were destroyed by the beetles. The plots were single rows of plants 16 feet long and there were 7 replicates.

In some tests on cucumbers in 1952 (experiment 6), all the materials gave fairly good control of the striped beetles, but NPD and A-42 were least effective. In tests on Hubbard squash at the same location (experiment 7), control was obtained with all the materials. In another experiment on cucumbers (experiment 8) most of the materials were effective, although NPD was inferior as in experiment 6 and malathion at 1% seems to have been too weak. In these experiments dead beetles were most numerous in the plots treated with parathion, EPN, aldrin, and dieldrin. The plots were 2 rows wide and 36 feet long and the treatments were replicated 5 times.

SUMMARY.—From 1945 to 1952 many of the new insecticides were tested in Ohio against the striped cucumber beetle, *Acalymma vittata* (F.), and the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* Barb.

Laboratory tests showed that many of the insecticides were very toxic to both species. The relative effectiveness of the better insecticides was difficult to determine in field tests because of the moving habits of the insects, but many of them gave good control when used at strengths of 1 or 2 per cent. The most effective materials were rotenone, lindane, EPN, dieldrin, aldrin, heptachlor, Bulan, and DDT.

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[3] 1,2-epoxy-4,5,6,7,8,8-hexachloro-4,7-methano-3a,4,7,7a-tetrahydroindane.

SCIENTIFIC NOTES

A Fortified Carrot Medium for Mass-Culture of the Oriental Fruit Fly and Certain other Tephritids

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In October 1949 the Department of Biological Control of the University of California set up a laboratory in the Hawaiian Islands to develop mass-culture methods for rearing certain of the most effective parasites of the Oriental fruit fly, *Dacus dorsalis* Hendel. The successful production of these parasites was contingent upon the development of a dependable medium of moderate cost for culturing the Oriental fruit fly larvae. The prerequisites for this medium were that it be nutritious, easy to handle, stable, cheap, and made of a commodity available in California throughout the year. The last requirement was necessary because the medium was to be developed for use in that State should the fly be found there.

After much experimentation a fortified carrot medium was developed which met all the desired qualifications. All of the *D. dorsalis* produced in the laboratory from February 1, 1951 to the termination of the project, July 1, 1951, were cultured on this medium.

The carrots were first washed and then shredded in a hand operated salad cutter after which they were reduced to a gravy-like consistency in a high speed blender with only enough water added to facilitate the blending action. The following ingredients were then blended into each 800 cc. of the carrot material:

1.04	grams Butoben (0.13%)
16	grams Brewers' yeast (Powdered) (2%)
15	cc. 2N HCl. (made by adding sufficient water to (164.6 cc. concentrated HCl to make one liter)

The Butoben (n-Butyl Parahydroxybenzoate, Merk.) was used to suppress the growth of undesirable molds and exotic yeasts. The Brewers' yeast served to compensate for inherent nutritional deficiencies in the carrots and to satisfy the dietary requirements of the Oriental fruit fly larvae. The function of the acid was to inhibit the development of bacteria.

The fortified carrot medium was very stable and could be prepared several days in advance if held in covered containers under normal refrigeration (38°–45° F.). Carrots are relatively inexpensive when purchased in quantity from a produce house. They may be stored in a "cold room" for a considerable period; however, carrots that are shriveled or show evidence of rot should be avoided.

The essential nutritive elements, such as wheat germ oil and cholesterol, the cost of which made the large scale use of the synthetic medium (Maeda *et al.* 1952) prohibitive, were supplied by the carrots.

Perhaps the most important single attribute of the carrot medium was its unique physical properties. There was on the surface of the medium a film of liquid of such consistency that hatching larvae could migrate freely to any part of the unit. This surface consistency was not found in the two next best media, the banana medium and the synthetic agar medium. The surface viscidity of these media was largely responsible for the mortality found among these first stage larvae.

The physical property of the carrot medium was singularly favorable also to the later stages of larval development. The larvae cannot feed vertically beyond their body length from the surface of the medium as they need continual access to a supply of air. Nevertheless the medium had to be poured into the units to a depth of not less than 1 inch to prevent significant desiccation of the material during the 6-day period of larval development. The carrot medium, however, tended to remain open to form tunnel-like channels behind the feeding larvae, particularly during the later stages of larval growth, and so permitted them to feed to the bottom of the medium. The larvae could thus gain access to the total nutrient content of the unit. In all the other media tried this was not the case, so the feeding was restricted to the upper stratum. Thus the depletion of rich nutritive values of the media was significantly increased by the repeated ingestion of the material within this limited zone.

Metal pans 13″×20″×3″ were used as culture units each containing 3200 cc. of medium. A culture unit in which 21,000 to 23,000 eggs were introduced would yield about 15,000 mature larvae. Nearly 80 per cent of the eggs were hatchable; 80 per cent of the young larvae reached maturity, and 95 per cent of these pupated and emerged as adult flies. Over 5,000,000 insectary reared *D. dorsalis* were utilized as test flies during the year ending July 1, 1951.

The fortified carrot medium produced strong healthy larvae that were resistant to disease resulting from the invasion of an undetermined protozoan species of the genus *Nosema*. This was also true of the synthetic agar medium and banana medium. This disease annihilated many cultures of larvae grown in squash or pumpkin media. This factor is of prime importance when the culture of larvae is carried on through many generations.

The fortified carrot medium was developed specifically for *D. dorsalis*, however it was used with some degree of success in culturing the larvae of *Ceratitis capitata*, the Mediterranean fruit fly. Although production of *C. capitata* was somewhat erratic it is felt the medium could be further modified to make it more suitable to the culture of this species. Glass dishes, 7″×7″×2½″, each holding 800 cc. of medium served as culture units. As many as 2500 mature larvae were obtained from a unit in which approximately 5000 eggs were introduced. Over 660,000 adult flies were produced during the first 3 months of 1951.

The larvae of the melon fly, *Dacus cucurbitae*, would not tolerate the presence of Butoben in the carrot medium and without this mold inhibitor the medium would scum over and deteriorate before the larvae could fully utilize it. This condition seemed to lower the vigor of the larvae to the point where they were unable to resist the invasion of the microsporidian, *Nosema*, so that entire stocks were wiped out by the disease.

The eggs of *D. curcurbitae* could not be obtained in a free state as were those of *D. dorsalis* and *C. capitata*. Cut sections of pumpkin, squash, or cucumber were exposed to large numbers of ovipositing flies. The larvae were allowed to feed until the sections were well consumed or until they had developed into the second stage at which time the sections along with the feeding larvae were placed in units of standard carrot medium without Butoben. Previous to the advent of *Nosema* and the high incidence of airborne spores in the laboratory, hundreds of thousands of insectary-reared flies were utilized as laboratory test insects in an insecticide toxicity evaluation program. It is possible that with further modification this medium might be rendered still more useful for the successful culture of *D. cucurbitae* even in the presence of *Nosema*.

The period of 2 years allotted this project expired before many of the subprojects could be completed. Thus several points of investigation were left with the final answers unresolved.

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Contaminants in Radioactive Allethrin Determined by Paper Chromatography[1]

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Since its synthesis and chromatographic purification were reported (Acree *et al.* 1954), C^{14}-allethrin (*dl*-allethrolone ester of *dl-cis, trans*-2-C^{14}-chrysanthemumic acid)[2] has been studied by the procedures reported by Winteringham (1952) without the atmosphere of nitrogen and washed paper, but the results differed somewhat from his. He described a reversed-phase paper method using ammoniacal ethanol for the separation of allethrin (Rf 0.40) from allethrolone and chrysanthemumic acid, which traveled together with Rf 0.89. He considered that these compounds are the hydrolysis products of allethrin and found that their mixture separated when it was chromatographed by a second procedure with acidified petroleum ether followed with ammoniacal petroleum ether.

When sufficient C^{14}-allethrin (25–100 μg.) was chromatographed by the reversed-phase procedure, in addition to the main zone B of C^{14}-allethrin (Rf 0.30), a trace of an unsuspected radioactive zone A at Rf 0.0 and a small radioactive zone C at Rf 0.84 were detected. Although the Rf values for dimerized allethrolone,[3] allethrolone, and 2-C^{14}-chrysanthemumic acid were found to be 0.78, 0.82, and 0.85, respectively, some doubt existed concerning the nature of zone C, because none of these compounds should have been present in C^{14}-allethrin owing to the method used for its purification.

The materials contained in zones A and C, separately sectioned from two unsprayed chromatograms of C^{14}-allethrin (200 μg.) were appropriately combined, transferred with ammoniacal 70% ethanol to fresh strips (Winteringham 1953), and chromatographed according to Winteringham's second procedure (1952). The results, although inconclusive, demonstrated that zone A remained at the origin and the presence in zone C of an unsuspected radioactive material at Rf 0.0, later found to consist of materials D and E.

Because of its importance to the metabolic fate of C^{14}-allethrin in insects, additional work was undertaken to determine the constituents of zone C. It was found that dimerized allethrolone produced double zones (Rf 0.4 and 0.89) when chromatographed with both the acidified and ammoniacal solvents, but only a single zone (Rf 0.89) with the latter solvent; that the chromatographic behavior of the radioactive materials D and E were comparable to that of contaminants contained in 2-C^{14}-chrysanthemumic acid; and that up to 75 per cent (determined radiometrically) of the latter volatilized when the chromatographic strips were dried for 1 hour in the hood to remove the acidified solvent.

The difficulties described above have been overcome, and an efficient qualitative analysis of zone C has been effected by the following procedures and checked with allethrolone, dimerized allethrolone, and 2-C^{14}-chrysanthemumic acid. Additional combined material from two unsprayed chromatograms (200 μg. C^{14}-allethrin) was transferred as already mentioned to a fresh strip, which was then sprayed with 1% aqueous ammonium chloride and dried. The material was chromatographed with ammoniacal isopropyl acetate in the manner described for the separation of *dl-cis* from *dl-trans*-chrysanthemumic acid (Acree & Babers 1954). The developed chromatogram demonstrated the presence of the unknown material D at Rf 0.0, the presence of small quantities of both *dl-cis* and *dl-trans*-2-C^{14}-chrysanthemumic acids, and a radioactive zone at Rf 0.98. This zone, in which allethrolone, dimerized allethrolone, and unknown E were found to travel, was sectioned from another unsprayed chromatogram, transferred to a fresh strip, and chromatographed with Skellysolve B saturated with concentrated ammonium hydroxide. This second chromatogram contained the zone of unknown radioactive material E at Rf 0.0. There was no indication of the presence of allethrolone, but a small quantity of dimerized allethrolone was present at Rf 0.89.

Thus it has been demonstrated that the reversed-phase chromatogram of C^{14}-allethrin with respect to ammoniacal ethanol contained trace quantities of five contaminants—one (A) at Rf 0.0 and a mixture of four (C) at Rf 0.84. This mixture, with respect to ammoniacal isopropyl acetate, consisted of an unknown D at Rf 0.0, both *dl-cis* and *trans*-2-C^{14}-chrysanthemumic acids, and a mixture at Rf 0.98. The last mixture consisted, with respect to ammoniacal Skellysolve B, of an unknown E at Rf 0.0 and dimerized allethrolone at Rf 0.89.

At present the most logical explanation for the presence of chrysanthemumic acid and dimerized allethrolone is that traces of allethrin are hydrolyzed during reversed-phase chromatography by the ammoniacal solvent. However, those products may be produced by some other reaction, such as photodecomposition, because zones A and C also have been observed on chromatograms prepared in the absence of ammonia.

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[1] Accepted for publication August 24, 1955.

[2] The radioactive C^{14} used in these investigations was obtained on authorization from the U. S. Atomic Energy Commission.

[3] La Forge *et al.* (1952) reported that chrysanthemumic acid and dimerized allethrolone are the products of *in vitro* hydrolysis of allethrin.

Substitution of Dehydrated for Fresh Carrots in Medium for Rearing Fruit Flies[1]

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A fortified fresh carrot medium developed by the University of California Agricultural Experiment Station (Finney 1953) has been used by the U.S.D.A. Fruit Fly Laboratory in Honolulu for rearing nearly 50 million experimental fruit flies during the last 4 years. This medium has been entirely satisfactory for the production of oriental fruit flies (*Dacus dorsalis* Hendel) and with slightly modified techniques it has also been useful for rearing large numbers of melon flies (*Dacus cucurbitae* Coq.) and Mediterranean fruit flies, *Ceratitis capitata* (Wied.).

The possibility that the irradiated fly technique used to eliminate the screw-worm maggot in Curacao (Bushland *et al.* 1955) may have application to fruit fly problems has stimulated renewed interest in mass rearing methods. If research now in progress in Hawaii indicates sufficient promise for the sterile fly procedure and large-scale tests are undertaken, the facility with which large numbers of fruit flies can be produced and their cost will become extremely important.

In experiments conducted early in 1955 dehydrated carrots were substituted for the fresh carrots used to prepare the fruit

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fly rearing medium. The oriental fruit flies produced on the dehydrated carrots proved to be remarkably uniform in size, the larval weight index (weight of 600 mature larvae) for 15 samples from more than 500,000 puparia varying only from 11.3 to 11.6 grams. Comparable indices for flies reared on the fresh-carrot medium varied from 9 to 12 grams. In other essential characteristics the two groups of flies were equivalent. The new dehydrated-carrot medium also improves Mediterranean fruit fly and melon fly culture. However, occasional difficulties in the production of the Mediterranean fruit fly are still encountered. After several trial runs demonstrated its suitability, the new dehydrated carrot medium was adopted as the standard in the fruit fly rearing room.

Heretofore the base medium was prepared by reducing fresh carrots to a pulpy mass by a time-consuming process of grinding them in a meat grinder and blending them in a Waring Blendor. Most of the juice extracted during this process was mixed back into the blended mass to obtain the desired consistency, but some of it was lost. The juice probably is rich in nutritive materials.

When dehydrated carrots are used, 1 part of milled dehydrated carrot powder is mixed with 7 parts of water. Before the water is added the powder is passed through a 28-mesh nylon screen to separate out the more granular particles. The fine-powder portion is used for oriental and Mediterranean fruit fly production. Melon fly larvae develop better in a medium prepared from the granular extract and they require a slightly drier medium than the other two species. Other nutrients and antibiotics may be added either before or after the water is added.

Substituting the dehydrated carrot medium for one prepared from fresh carrots has reduced the cost of raw materials by more than one-half and it permits exact moisture control. It has also eliminated the labor formerly required to grind and blend the fresh carrots and the cost of frequent repairs to high-speed food blenders. The success obtained with the dehydrated carrot medium suggests the possible usefulness of other dehydrated products and packaged powdered carrot formulas containing all necessary added nutrients and antibiotics which can be transported to and stored in remote island areas where pilot tests of the sterile male technique may be undertaken.

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Residues on Forage, in the Soil, and in Milk Following Pasture Treatment with Granulated Dieldrin[1]

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In recent years there has been considerable experimental work with granulated insecticides to control various insects. The granules can be applied like dusts, but being heavier they drop through the foliage to the soil surface. There is a minimum loss of material, since granules drift much less than dust. It is generally believed that the residue problem will be lessened by the use of granules.

During the summer of 1954 an experiment was conducted at Beltsville, Md., to obtain information on residues on the forage or in the soil of pastures treated with granulated dieldrin, and on the amount of the insecticide appearing in the milk of cows allowed to graze in such pastures. Two 3-acre pastures containing mostly bluegrass were selected for the tests. One was treated with 0.5% dieldrin granules at 0.53 pound and the other with 2.5% granules at 4.18 pounds of actual dieldrin per acre. Both formulations were on 30/60 mesh attapulgite. The materials were applied on June 14, with a fertilizer distributor that treated a 10-foot strip.

Table 1.—Dieldrin residues on the forage and in the soil of pastures treated with granulated dieldrin at 0.53 or 4.18 pounds per acre.

Days After Treatment	Parts per Million on Forage		Pounds per Acre in Soil[a]	
	4.18 Pounds	0.53 Pound	4.18 Pounds	0.53 Pound
1	30.6	Lost	2.65	0.38
2	7.5	3.7	—	—
7	30.8	2.7	3.92	.70
14	20.6	4.6	2.97	.34
21	11.4	2.2	—	—
28	6.4	2.8	2.45	.47

[a] Corrected for apparent dieldrin in check soil, equivalent to 0.32 pound per acre.

At regular intervals samples of the forage and soil were taken for residue analysis. On each date five forage samples, each consisting of the vegetation cut from 1 square yard, were taken from each treated pasture and from an adjoining untreated pasture. The forage was air-dried and stored in paper bags. The five samples were later combined into a composite sample. Soil samples were taken to a depth of 3 inches with a soil auger having a 1-inch inside diameter. A composite sample made up of 150 cores (50 per acre) was taken in each pasture. The soil was stored in half-gallon fruit jars until analyzed.[2] Table 1 shows the results of the analyses.

In each of the treated pastures four cows were allowed to graze, two during the first 14 days after application, one from the 4th to the 12th day, and one from the 15th to the 22nd day. Milk samples were taken from each cow and analyzed for dieldrin. The results are in table 2.

Table 2.—Excretion of dieldrin in the milk of cows grazing in pastures treated with granulated dieldrin.

Dieldrin Dosage and Cow No.	Days After Treatment	Number of Samples	Dieldrin	
			P.p.m.[a]	Milligrams
0.53 pound per acre				
1	1–14	8	0.18	1.4
2	1–14	8	0.21	1.8
3	4–12	4	0.15	1.5
4	15–22	3	0.11	1.2
4.18 pounds per acre				
5	1–14	8	0.96	9.3
6	1–14	8	0.96	8.7
7	4–12	4	0.52	7.9
8	15–22	3	0.04	0.5

[a] In fat-corrected milk.

Each of the cows grazing during the first 14 days after application in the pasture treated with 4.18 pounds of dieldrin had 0.96 p.p.m. of dieldrin in the milk. These amounts of residue are comparable to those found in the milk of cows fed hay contain-

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[2] R. D. Chisholm, of the Moorestown, N. J. laboratory of the Entomology Research Branch, made the soil analyses.

ing 0.4 p.p.m. of dieldrin (Ely *et al.* 1954). Not more than 0.21 p.p.m. of dieldrin was excreted in the milk of the cows grazing in the pasture that received 0.53 pound per acre.

More than 60 per cent of the dieldrin was recovered in the soil samples taken 1 day after treatment. However, sufficient material adhered to the foliage to show considerable residues from both dosages, and cows grazing in these pastures excreted dieldrin in the milk for 15 to 22 days after application.

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Recently Discovered Primary Hosts of the Foxglove Aphid[1]

H. E. WAVE,[2] W. A. SHANDS,[2] and G. W. SIMPSON[3]

Common foxglove, *Digitalis purpurea* L., was named by Patch (1928) as the primary host of the foxglove aphid, *Myzus solani* (Kltb.) (=*pseudosolani* Theob.), an important potato-infesting species, in Maine. Lambers (1947) stated that in Europe aphids of this genus, which he called *Aulacorthum*, overwinter as eggs on various plants. Heinze & Profft (1940) reported that in Germany foxglove aphids overwinter as eggs on forget-me-not, *Myosotis alpina* Lapeyr., sheperds-purse, *Capsella Bursa-pastoris* L., and possibly alfileria, *Erodium cicutarium* (L.) L'Her.

Since careful observation indicated an almost complete absence of foxglove in the potato-growing districts of Maine, it seemed likely that other primary hosts of the foxglove aphid must exist there, in view of the large numbers that initially infest potatoes each year. A series of surveys were made in central and northern Maine, beginning in 1943 and continuing for several years, principally at the time of the spring migration. Infestations of the foxglove aphid were observed frequently on hemp nettle, *Galeopsis Tetrahit* L., but these infestations were thought to have been started by spring migrants. Although snakehead, *Chelone glabra* L., proved to be an excellent host throughout the growing season under cage conditions, naturally occurring colonies were never found on the plant.

In 1953 a more intensive study was started in Maine and New Hampshire to determine and delimit geographically the primary hosts responsible for the initial infestations of this aphid on potatoes in northeastern Maine. Indigenous foxglove was found to occur very rarely either in Maine or in New Hampshire.

On May 20, 1954, mature stem mothers of the aphid with progeny were found on hemp nettle near Ashland, Maine. Closely ensuing observations revealed that the plant was similarly infested in a number of locations near Ashland and Masardis. Also one infestation was found on evening primrose, *Oenothera biennis* L., near Ashland. The infested evening primrose plants were in a mixed stand containing hemp nettle. All infested hemp nettle plants were growing in or adjacent to undisturbed fields that had been planted to potatoes in 1953.

Large spring populations developed on hemp nettle in several of these places, and spring migrants began to mature about June 8. The first spring migrant caught in the vicinity of Presque Isle in 1954 was taken on June 12 from one of several aphid traps in that agricultural district that were being emptied daily. The closeness of these two dates suggests the possibility that the migrants caught in the traps developed on local host plants.

In the fall of 1954 oviparous forms and wingless males developed on hemp nettle in central Aroostook County. Mature oviparae and recently deposited eggs were first observed on this plant on October 20. Hemp nettle was more heavily and generally infested than at the same time in 1953.

On October 22, 1954, hawkweed, *Hieracium* sp., growing in an old apple orchard near Smyrna Mills was found to be heavily infested with the foxglove aphid. The infestation on individual plants included viviparae, oviparae, males, mating pairs, ovipositing adults, and recently deposited eggs. Later, hawkweeds of several species were found to be generally infested with this aphid in practically all places in Aroostook County where observations were made. Generally plants in rank-growing grass, hedgerows, and similarly protected places were most heavily infested. The infested environments ranged from borders of potato fields to places 10 or more miles from potatoes. The presence of old, dead, parasitized viviparae on infested plants farthest removed from potato fields strongly indicated that the aphid utilized hawkweed as a host during the summer. Although Patch (1928) indicated that fall migrant forms develop late in the summer, Lambers (1947) reported that true fall migration had not been recorded. Observations by the writers show that winged forms seldom develop on potatoes or on other secondary hosts during late summer or fall.

On November 23, 1954, a survey was made to determine the abundance of aphid eggs on hawkweed at 11 widely separated stations in northeastern Maine. Even though the size of the sample examined at each station was small, eggs were found on 10 to 33 per cent of the plants at the six stations found to be infested.

Studies in the spring of 1955 showed that eggs of the foxglove aphid overwintered on hawkweed and that the aphid bred throughout the spring on the plant at all the sampling stations.

These observations show that hemp nettle and several species of hawkweed are primary hosts of the foxglove aphid in northeastern Maine. Hawkweed is a perennial plant, which is abundant in grassland, pastures, old orchards, cut-over land, along roadsides, and to some extent in cultivated fields. Hemp nettle is an annual that grows generally and in abundance in crop land and other exposed environments. Of the two plants, hawkweed occurs in much greater abundance and over more extensive areas because it withstands competition from other plants far better than hemp nettle. Furthermore, hemp nettle may not serve as a primary host as generally as hawkweed. Although spring forms of the aphid develop on hemp nettle, we have not determined whether the immature stem mothers initiating the spring colonies hatched from eggs on adjacent, dead hemp nettle or on nearby hawkweed.

Patch (1928) listed the hawkweed, *Hieracium aurantiacum* L., as an acceptable host of this aphid in Maine. A search of her records disclosed that only single specimens were collected from plants at Orono and Freeport. Although she reared several generations of this aphid, taken in June from foxglove at Freeport, on *aurantiacum*, she evidently did not consider it as a primary host, nor did she attach much importance to it as a secondary host.

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Activated-Carbon Filter for Purification of Air for Rearing Citrus Red Mite[1]

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All attempts to rear the citrus red mite, *Metatetranychus citri* (McG.), in the laboratory at Whittier, California, were unsuccessful until a large amount of fresh air was circulated in the rearing room (Munger 1955). This fresh-air requirement indicated that impurities may have been present in the laboratory air. Although quantities of mites could be reared continuously in this way, the need for conditioning the introduced air made it difficult to maintain controlled temperatures and humidities, and other ways of removing air contaminants were sought. Activated-carbon filters have been used for air purification in institutions, storage chambers, greenhouses, and other situations. These uses suggested trying them in the closed chambers in which mites were to be reared.

In a preliminary experiment, a tightly closed cabinet having a volume of approximately 6 cubic feet was provided with a filter. The filter consisted of three cylindrical, perforated adsorption canisters, each containing 1½ pounds of granular activated carbon in a uniform bed thickness of ¾ inch. Air from the cabinet was forced through the filter at a rate equivalent to 735 times the volume of the cabinet each hour. The mites thrived within this cabinet at approximately 77° F. and 60 to 70 per cent relative humidity from November 16, 1954, to April 11, 1955, when the experiment was ended. Apparently, substances that were toxic to the mites were removed from the air by the carbon.

With a view to simplifying the equipment and to making possible simultaneous tests in a number of uniform-sized cabinets, a test was made with three cabinets in a room provided with a carbon filter running continuously. The room had a volume of 1148 cubic feet and the filter an hourly capacity approximately five times the volume of the room. Different amounts of air, the equivalent of approximately 34, 4, and 0 times the volume of the cabinet, were passed through the cabinets from the room each hour. Though one cabinet had no forced air exchange, some air from the room entered when doors were opened three to four times a week to change the thermograph chart and to make mite population counts; there was also a small leakage around the doors. Similar leakage occurred in the other cabinets but was negligible relative to the amount of forced air circulation.

On March 20, five newly emerged female mites, reared in the laboratory on lemons, were placed on each lemon of five groups of 10 lemons. Precautions were taken to have all mite sources (individual lemons) nearly equally represented in the five groups. A group of lemons was placed in each of the three cabinets, one group was placed in the rearing room which received the equivalent of 2½ changes of air from outdoors each hour, and another in the cabinet that was used in the preliminary experiment. All five groups were held at 77° F. and 60 to 70 per cent relative humidity. Eggs were counted and removed from all the lemons at frequent intervals and the number of females present was recorded. The results of this experiment are shown in table 1.

By far the smallest number of eggs were produced in the cabinet with no filtered air circulation. This decrease was associated with higher mortality of the females. Egg production was just as high in the cabinet with only four air changes per hour as in the cabinets receiving much more filtered air or in the room with fresh air from outdoors.

In the production of mite material for biological and toxicological work, purification of the air in the rearing room has been substituted for the introduction of quantities of fresh air since April 20, 1955, when a carbon filtering unit with an hourly capacity of 6½ times the volume of the room was installed. To the present time, a period of 13 weeks, the mites have thrived fully as well as previously.

The results show that purification of air with activated carbon can be substituted for circulation of fresh air in the laboratory rearing of the citrus red mite. This has considerable advantage when one is working under controlled temperature and humidity, because the same air is used over and over and consequently much less conditioning with heat or moisture is required. Purification of air may be important in the laboratory rearing of other organisms.

Table 1.—Number of eggs produced in 20 to 21 days by 50 female mites on 10 lemons under various conditions at 77° F. and 60 to 70 per cent relative humidity.

Condition of Test	Number of Air Changes per Hour	Number of Eggs Laid	Number of Females Alive After 10 Days
Cabinet:			
Air through attached filter	735	2847	21
In filtered room	34	2949	22
	4	3119	18
	0	1733	12
Rearing room, air from outdoors	2½	2660	20

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Tests with Self-Treating Devices for the Control of Lice on Cattle in Mississippi[1]

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The increasing interest in self-treating devices for the control of ectoparasites of cattle prompted the field test, results of which are presented in this paper, to determine the feasibility of using back-rubbers for the control of lice on cattle in Mississippi. Rogoff & Maxon (1952) have shown that back-rubbers effectively controlled horn flies on cattle in South Dakota. Lindquist & Hoffman (1954) have demonstrated that this type of device worked equally as well in Oregon for the control of horn flies in that state. The success with horn fly control led Hoffman (1954) to investigate the use of this device for the control of lice infesting feed-lot cattle. The results obtained from his field trials in Oregon showed that chlordane-impregnated back-rubbers gave satisfactory control.

This investigation, conducted in Mississippi during the winter of 1954–55, includes observations made on beef cattle only. The periodic examination of a number of dairy herds near State College revealed that lice infestations were negligible. Out of a total of six dairy herds examined over a period of 3 months, one herd was found to be lightly infested.

The following three species of lice were taken from the test groups of cattle: *Solenopotes capillatus* (Enderl.), *Linognathus vituli* (L.) and *Bovicola bovis* (L.). A few specimens of the last named species were taken only from two animals at Stoneville, Mississippi. The observed specimens of *Solenopotes capillatus* far outnumbered those of *Linognathus vituli*.

Although test groups usually consisted of mixed Hereford and Aberdeen-Angus cattle, only the Hereford animals were examined for the presence of lice. Because lice on the Aberdeen-Angus group were difficult to detect, the sampling of this breed for lice was not attempted.

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Table 1.—Self-treating devices for the control of lice on cattle in Mississippi.

Location and Feed Lot Size	Herd	Material and Per Cent Applied	Number of Animals Sampled	Pretreatment Counts No. of Animals in Each Rating Group[a] O	L	M	H	Per Cent Reduction After (days) 8	21	30	Increase or No Reduction; No. of Animals in Each Rating Group[a] After (Days) 21 O	L	M	H	57 O	L	M	H	76 O	L	M	H
State College	1	Chlordane, 5	9	—	3	4	2	100	—													
Lots approx.	2	Chlordane, 5	6	—	3	3	—	—	100													
300×100 ft.	3	Toxaphene, 5	10	—	—	4	6	99[b]	—													
	4	Check	6	—	4	2	—				—	2	—	4								
Stoneville,	5	Chlordane, 5	5	2	3	—	—		100	100												
Lots approx.	6	Chlordane, 5	5	1	4	—	—		100	100												
400×100 ft.	7	Check	5	1	2	1	1				—	—	2	3								
	8	Toxaphene, 5	8	—	4	1	3				1	2	2	3					—	3	1	4
	9	Toxaphene, 5	10	—	5	5	—								—	5	5	3				
West Point Range lots	10	Lindane, 0.3 +Toxaphene, 5	10	—	5	5	—								—	1	4	5				
10 to 200 acres	11	Lindane, 0.3 +Toxaphene, 5	10	—	5	3	2								—	—	2	8				
	12	Check	8	—	3	4	1								—	2	3	3				

[a] Denotes louse infestation as follows: 0, no lice; L, light, occasional louse observed; M, medium, 5 to 10 lice per examination area: H, heavy, over 11 lice per examination area.

[b] Estimated. One animal was observed with a few lice around eyes and nose.

MATERIALS AND METHODS.—The self-treating devices employed were essentially the same as those used by Hoffman in Oregon. They were constructed so that a center pole supported two 3-strand barbed wire cables attached to the post at a height of approximately 5 feet from the ground level. The two cables were extended in opposite directions and were secured to short posts set at points in the ground approximately 9 feet from the center pole. Three layers of burlap sacks were wrapped around the cables and were secured with heavy cord. Three layers of the sacks also were wrapped around the lower part of the center pole to provide additional rubbing surface.

At Stoneville and State College, where 10 animals were maintained in each feed lot, one back-rubber was assigned to each lot. At Prairie Queen Farms near West Point, where the cattle were on lots ranging in size from 10 to 200 acres, the number of back-rubbers per lot varied. An attempt was made to make one rubbing device available for 50 or less head of cattle.

Three insecticide emulsions were used. At Stoneville, a 5% concentration of chlordane emulsion in No. 2 fuel oil was used; at State College, a 5% concentration of chlordane and a 5% concentration of toxaphene in No. 20 motor oil were tried; at Prairie Queen Farms, toxaphene at a concentration of 5% and a combination of a 5% toxaphene and a 0.3% lindane in No. 2 fuel oil were tested.

Initial impregnation of each back-rubbing unit consisted of 1½ gallons of the finished material. Subsequent applications of the material to each back-rubber were made once every 2 weeks at the rate of 2 quarts per rubbing unit.

Finding a proper method of sampling the louse population was one of the most difficult problems encountered. Whatever procedure is selected is always subject to fairly obvious errors. Some of these errors arise from the difficulty encountered in attempts to count the lice on the animals complicated by the natural tendency of some lice to clump themselves together in dense groups. Also, infestations are not uniform over the body, and frequently the time that the examiner must spend in arriving at an estimate at several points on the animal's body takes so long that only a comparatively few animals can be accurately examined. A rating system was used in this study to denote the severity of louse infestations. An occasional louse observed is rated as *light*, 5 to 10 lice per examination area is considered *medium* and over 11 per examination area is rated as *heavy*. The parts of the animal's body that were examined with particular care before extablishing a rating were the head, neck, dewlap and shoulders.

Observations were made on the test groups of cattle at the following locations (Table 1): (1) State College, groups 1, 2, 3 and 4, 10 animals per group, one-year-old Hereford and Angus steers and heifers; (2) Stoneville, groups 5, 6 and 7, 10 animals per group, one-year-old Hereford and Angus steeers; (3) Prairie Queen Farms, group 8, 112 Hereford cows, age 2 to 5 years; group 9, 132 Hereford and Angus calves, age 6 months; group 10, 200 Hereford and Angus heifers, age 8 to 18 months; group 11, 90 Hereford and Angus steers, age one year; group 12, 10 Hereford and Holstein steers, age one year.

RESULTS.—The use of chlordane impregnated back-rubbers located on feed lots (300×100 feet) at Stoneville resulted in adequate control of lice on cattle within a reasonably short time (Table 1). Satisfactory control also resulted under similar conditions from the use of chlordane- and toxaphene-impregnated back-rubbers at State College. Untreated cattle on feed lots at both areas showed an increase in lice during the test period. The use of toxaphene and toxaphene-lindane impregnated rubbers on range lots (10 to 200 acres) failed to control lice on cattle at Prairie Queen Farms. It should be noted that the back-rubbers were tested under two entirely different conditions. At one location, Prairie Queen Farms, they were set up on large range lots, whereas at Stoneville and State College they were maintained on small feed lots. For this reason, an evaluation of the effectiveness of the toxaphene and toxaphene plus lindane on range lots cannot be compared with the effectiveness of chlordane on small lots. However, one might logically assume that chlordane-impregnated back-rubbers also would fail to control lice on range lot cattle. Observations on the cattle receiving treatments at State College and at Stoneville unavoidably covered a short period of time because they were turned on pasture during the second week of March, at which time examination for lice had to be terminated.

SUMMARY.—During the winter of 1954–55, back-rubbers impregnated with a 5% concentration of chlordane in No. 2 fuel oil or motor oil were found to control sucking lice on beef cattle confined in small feed lots. A 5% concentration of toxaphene gave effective control, also, under the same conditions; but the performance of this material needs further testing, because the results were taken from only one group of experimental animals.

Back-rubbers located on large range lots, impregnated with a 5% concentration of toxaphene or a combination 5% toxaphene plus a 0.3% concentration of lindane in No. 2 fuel oil did not control sucking lice on beef cattle.

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A Field Test of Malathion to Control Sarcoptic Mange of Hogs[1]

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Since many workers have reported the effectiveness of malathion in the control of mites attacking various crops, it would be desirable to know the effectiveness of the material in the control of the various zoophagous mites. With this in mind an abbreviated field test of the efficacy of malathion in the control of the hog mange mite, *Sarcoptes scabiei suis* (Gerlach), was carried out when the opportunity presented itself during the summer of 1955.

PRELIMINARY TOXICITY TRIAL.—In order to be certain of the effects of malathion on swine, preliminary tests were made on the Bert Eason farm near Scranton, Iowa. Twelve crossbred hogs (Duroc, Yorkshire, Landrace) were selected from Mr. Eason's 4-month old herd. All animals weighed 130 to 140 pounds.

The 57% malathion emulsifiable concentrate was used as the insecticide. Tide was used at the rate of ½ pound per 25 gallons of water as a wetting agent. One quart of spray solution was applied to thoroughly wet each animal. Four hogs were sprayed with each of the malathion concentrations; 0.5 per cent, 1 per cent, and 2 per cent. All spraying was done with a 3-gallon compression sprayer. During the following 2 weeks, the treated animals exhibited no gross symptoms of toxicity or skin irritation.

MANGE CONTROL TEST.—Since the results of the preliminary work showed that a concentration of up to 2% malathion could be used with safety on swine, a herd of 30 mangy hogs on the Dwayne Hill farm at Ellsworth, Iowa, was selected for treatment. These hogs were from 7 to 9 weeks old, but weighed only approximately 25 pounds.

The diagnosis of mange was made by observation of the gross skin lesions and by microscopic examinations of skin scrapings. Animals were heavily infested with the sarcoptic mite.

The 30 swine were divided into 3 lots of 10 each. Those in lot 1 were sprayed with 0.5% malathion concentration, those in lot 2 with a 1% malathion concentration, and those in lot 3 were left unsprayed as controls. The spray solutions were made by mixing 57% malathion emulsifiable concentrate with water. Tide, at the rate of ½ pound per 25 gallons of water, was added as a wetting agent.

The spraying was done with a tractor mounted power-take-off unit operating at 50 pounds pressure. Approximately 1 quart of spray was applied to each animal. The bedding and walls were sprayed at the same time in order to prevent reinfestation of the swine from infested quarters.

The swine were observed immediately after spraying and there were no apparent ill-effects. Subsequent observations were made as follows:

Third day—The swine in lots 1 and 2 showed less evidence of skin irritation by a decreased amount of rubbing and scratching. There was no apparent evidence of toxicity. Swine in lot 3 were unimproved.

Eighth day—The clinical picture of all groups was nearly the same as the third day.

Fifteenth day—There was a marked improvement in general condition of the swine in lots 1 and 2 as evidenced by an improved appearance of the skin, less rubbing and scratching, and an obvious gain in weight. The swine in lot 3 were unimproved.

Nineteenth day—The skin of the swine in lots 1 and 2 had returned to a normal condition. These animals made a similar response throughout the observation period despite the difference in concentrations of the spray. The swine in lot 3 appeared to be in the same condition as at the start of the observation period with severe mange lesions. Skin scrapings from these pigs showed the continued presence of sarcoptic mites.

SUMMARY.—Field tests of malathion sprays on hogs evidenced no gross symptoms of toxicity or skin irritation when applied at 0.5, 1.0, and 2.0 per cent concentrations.

Hogs severely infested with the mange mite *Sarcoptes scabiei suis* were sprayed with 0.5 and 1.0 per cent concentrations of malathion. Both concentrations eliminated the mangy condition from the treated animals in 19 days.

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Ufens niger (Ashm.), an Egg Parasite of the Geminate Leafhopper[1]

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In the spring of 1954 *Ufens niger* (Ashm.) was found to be parasitizing eggs of the geminate leafhopper, *Colladonus geminatus* (Van D.), at Logan, Utah. This parasite was detected during life-history studies of the geminate leafhopper, a primary vector of the western X-disease virus in stone-fruit trees.

The eggs parasitized were laid the previous autumn by leafhoppers reared in the greenhouse and then caged on outdoor mazzard seedling cherry trees. The leafhopper eggs were inserted into the leaf tissues from October until December, as long as there were any leaves on the trees. On April 2 the leaves of one small seedling were brought into the laboratory. Leafhopper nymphs hatched from April 5 to 10, and adult parasites were found to be emerging from April 8 to 10.

Leaves from a second cherry tree were brought into the warm laboratory on April 13, by which date leafhopper hatching had begun out-of-doors. Forty-nine adult parasites emerged from April 14 to 21. Then the leaves were fumigated and more adults of *U. niger* were dissected, dead, from leafhopper eggs.

Ufens niger was first described in 1888 by Ashmead as *Trichogramma nigrum* Ashmead. He based his species on two specimens, and did not give any information as to the date and locality of collection. It was redescribed by Girault in 1911 as *U. niger* (Ashm.) from specimens collected at Centralia and Mattoon, Ill., Salisbury, N. C., and Washington, D. C. Girault later (1914) reported collecting specimens from leafhopper eggs sent to him from Puerto Rico in 1912. Gibson reported finding *U. niger* parasitizing the eggs of the sharp-headed leafhopper, *Draeculacephala mollipes* (Say), in Florida.

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[1] Accepted for publication November 17, 1955.

[2] In cooperation with the Utah State Agricultural College and Experiment Station, Logan, Utah.

Common Names of Insecticides
Supplement to List in February 1955 Issue of This Journal

H. L. HALLER, *Crops Research, Agr. Res. Serv., U.S.D.A.*

At the 1955 meeting of the Entomological Society of America the Committee on Insecticide Terminology approved a supplemental list of Common Names of Insecticides. This list, given below, includes two of the changes indicated in the June 1955 issue (p. 345), but "Sulphenone" has been restored as in the original list, since the Stauffer Chemical Company has now acquired the right to this name for R-242.

NAME TO BE USED	DEFINITION	OTHER DESIGNATIONS THAT HAVE BEEN USED
Am. Cyanamid 3911	0,0-diethyl S-ethyl mercaptomethyl dithiophosphate	
Am. Cyanamid 4124	0-(2-chloro-4-nitrophenyl)-0,0-dimethyl thiophosphate	isochlorthion
Am. Cyanamid 12013	0,0-diisopropyl S-isopropyl mercapto-methyl dithiophosphate	
Bayer 28/63	[2-(diethoxyphosphinylthio)ethyl]ethyl-methyl sulfonium methyl sulfate	
Bayer 16259	0,0-diethyl-S-(4-oxo-benzotriazino-3-methyl) phosphorodithioate	a benzotriazine derivative of an ethyl dithiophosphate
Bayer 17147	0,0-dimethyl-S-(4-oxo-benzotriazino-3-methyl) phosphorodithioate	a benzotriazine derivative of a methyl dithiophosphate
Chlorthion	0-(3-chloro-4-nitrophenyl)-0,0-dimethyl thiophosphate	Bayer 22/190
DDVP	dimethyl 2,2-dichlorovinyl phosphate	
Dow ET-14	0,0-dimethyl 0-2,4,5-trichlorophenyl phosphorothioate	
Dow ET-15	0-methyl 0-2,4,5-trichlorophenyl phosphoramidothioate	
Karathane	dinitro(1-methylheptyl)phenyl crotonate	
methyl demeton	mixture of 0,0-dimethyl-S-ethylmercapto-ethyl and 0,0-dimethyl-0-ethylmercaptoethyl thiophosphates	
ovex	*p*-chlorophenyl *p*-chlorobenzenesulfonate	Ovotran K-6451
Rohm & Haas FW-152	1,1-bis(*p*-chlorophenyl)2,2-dichloroethanol	
Rohm & Haas FW-293	1,1-bis(*p*-chlorophenyl)2,2,2-trichloroethanol	
Shell OS 1836	diethyl 2-chlorovinyl phosphate	
Shell OS 2046	1-carbomethoxy-1-propen-2-yl dimethyl phosphate	
Sulphenone	product containing *p*-chlorophenyl phenyl sulfone and related sulfones	R-242

EDITORIAL
Effective Communication

Scientific journals are the circulatory system for the ideas of science according to Raymond B. Fosdick. It is largely through them that science advances, for scientific growth is the result of exchange of ideas between laboratories and groups throughout the World. The volume of "blood" circulated annually through the arteries of science is staggering. Recently, the editor of one of our most important abstracting journals estimated that approximately half a million scientific papers were published each year. Biological abstracts alone, annually prints 30,000 to 35,000 references and our own publication, the INDEX TO THE LITERATURE OF AMERICAN ECONOMIC ENTOMOLOGY, fills more than 300 pages a year.

What is the effect of this tremendous output from scientists and more particularly from entomologists on brilliant young minds seeking a place in the field of science? Can we compete with the current glamour of physics and engineering? Our recent celebration of 100 years of professional entomology increased the awareness of the tremendous progress in entomology but the more serious students are looking at our publications to see if we are continuing to progress. What impression does our JOURNAL give?

How do scientists in other fields regard our JOURNAL? Most critics will agree that its reputation is good and is getting better but improvements are still possible and constant vigilance is needed. Publication in the JOURNAL must remain an award for merit, not a right purchased by payment of dues.

The Entomological Society of America is most fortunate to have as editor one who tries to use good common sense in making continual improvement in the JOURNAL. His willingness to devote countless hours to the editorial task has contributed greatly to the increased stature which the JOURNAL OF ECONOMIC ENTOMOLOGY enjoys.

What other improvements may be made in the quality of the JOURNAL? Would a revision of the current "Publications Policies and Manuscript Rules and Suggestions for the JOURNAL of ECONOMIC ENTOMOLOGY" to provide more detailed instructions for authors, help produce better, more uniform manuscripts? Should use of a standard set of abbreviations for publications be mandatory? Is the current editorial policy satisfactory?

Dr. Robert C. Miller, speaking at the third annual Conference on Scientific Editorial Problems stated, "If you are going to maintain specific standards, tell the author what your standards are. . . . As a matter of fact

most things that most editors insist on can be spelled out on one mimeographed page, which can be supplied to a prospective author on request, or sent to him with the return of an unsatisfactory manuscript, with the suggestion that he comply. This avoids a great deal of argument, debate and needless correspondence and puts the editor in a position that can be defended by logic instead of *force majeure*." Brief instructions are given in each JOURNAL, should a more complete set be provided authors?

A hasty examination of a recent number of the JOURNAL revealed five different abbreviations for the word "agriculture" in references cited by different authors. Four abbreviations were used for "experiment," many similar examples could be given. To obtain greater uniformity should the authors be required to conform to the abbreviations listed in some standard reference such as "Abbreviations Used in the Department of Agriculture for Titles of Publications" (USDA Misc. Pub. No. 337)? Many societies have such a requirement and adhere strictly to it.

Should the editor and the editorial board be more rigid in their interpretation of the current policy which states, "Papers acceptable for publication shall in general consist of progress or final reports on original biological or biochemical research and its application in the field of applied entomology and closely related fields, including quarantine activities."? Many authors have submitted review articles for publication in the JOURNAL and suggested that a liberal interpretation of the publication policy be followed. Is such a policy change desirable? The writer believes that the ANNUAL REVIEW OF ENTOMOLOGY has been established for this purpose and a rigid interpretation of the policy of the JOURNAL should be followed.

D. E. HOWELL,
Oklahoma A. & M. College, Stillwater

BOOK REVIEWS

METHODS OF PLANT BREEDING (Second Edition), by Herbert Kendall Hayes, Forrest Rhinehart Immer and David Clyde Smith. McGraw-Hill Book Co., Inc., 330 West 42nd St., New York 36, N. Y. 1955. 9⅜×6⅜ inches, ix+551 pages, 103 tables, 64 figures. Price $8.50.

The second edition of this outstanding publication has been revised so as to incorporate important contributions that have accumulated in the field of plant breeding and crop improvement since the first edition was published in 1942. Of particular immediate interest to the entomologist is the addition of a chapter on Breeding for Insect Resistance. Plant breeders and geneticists working with the more important crop plants are giving an increasing amount of attention to resistance and tolerance to insect attack and are making it an integral part of varietal improvement programs. It is the expressed opinion of workers in this field of research that varietal improvement and stability in crop production in the future will be enhanced to a large extent through higher levels of resistance to diseases and insects. The authors emphasize the necessity for fundamental training in the biological sciences and regard a knowledge of plant pathology and entomology to be highly essential in plant breeding.

The forepart of this book deals to a large extent with genetic principles and their application in plant breeding methods. A knowledge of the modes of reproduction and the techniques used in breeding work is fundamental to an understanding of the various facets of varietal improvement. The section on Centers of Origin of Important Cultivated Plants, quoted primarily from Vavilov, is of interest in several phases of entomology, particularly biological control, taxonomy and distribution and varietal resistance. The authors have emphasized the importance of the biological interrelation between host and parasite and the development of biotypes or genetic strains that are able to persist successfully on different strains of plants. Similar genetic problems are involved in toxicological research.

The special chapter on Breeding for Insect Resistance presents discussions on a group of insect pests which are receiving considerable attention in varietal improvement programs. General information on the biology of the various species on their respective hosts is presented. An examination of the references cited will readily acquaint one with detailed information on the research activities in the field of insect resistance and its application. In addition to this chapter the literature on inheritance of plant resistance to some of the important species is discussed in the chapters dealing with Inheritance in Small Grains and Flax, and Inheritance in Maize, and references to insect pests are also made in the chapters on Cotton and Sorghum Breeding, Forage Crop Improvement, and Breeding Other Cross-pollinated Plants.

Several chapters are devoted to a description of testing techniques and statistical methods with appendix tables commonly used in connection with analyses of data obtained in breeding and testing programs. Similar methods are equally well adapted to a wide variety of entomological research problems.

This book is an important step forward in giving recognition to the progress that has been made in the biological approach to insect control and for stimulating increased activity in this field of research. It is highly recommended to students and investigators interested in the biology of insect control.

F. F. DICKE

OFFICIAL METHODS OF ANALYSIS OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 8th Ed., 1955. Published by the Association of Official Agricultural Chemists, P. O. Box 540, Benjamin Franklin Station, Washington 4, D. C. 1008 pp. Price $12.00 domestic; $12.50 foreign.

The purpose of this book is to present those standardized analytical procedures that have been demonstrated to be reliable, that have convenient practical application, and that at the same time give reproducible results in the hands of professional analytical chemists. This edition shows a substantial advance toward simplification of methods, which is made possible by developments in instrumental technique and by the extension of such techniques as chromatography to the isolation of substances from materials refractory to other means of separation.

As pointed out in the preface, one of the deficiences of this book is the lack of specific methods of analysis of spray residues of several of the new pesticides. Only two methods in this category have been adopted by the Association during the past five years —methods for mercury and for parathion (although adoption of certain others is apparently in near prospect). New methods of analyzing formulations of warfarin, potassium cyanate, 2,4-D, 2,4,5-T and organic thiocyanate have been introduced.

This book remains the most valuable single source of information on the analysis of pesticides and their residues on fruits and vegetables and in milk and animal tissues and is indispensable to the chemist engaged in this work.

R. C. ROARK

ANALYSIS OF INSECTICIDES AND ACARICIDES, by Francis A. Gunther and Roger C. Blinn. 696 pp. Chemical Analysis, Vol. VI. Interscience Publisher, Inc., New York, N. Y.

This book is an excellent introduction to the study of pesticide chemical residues. The concepts proposed in the first section (nine chapters) of the book may well apply to any pesticide residue. The authors strongly express their conviction that the study of residues is one of the primary steps in development of an insecticide or acaricide and that methods of study of residues should receive consideration in planning of experiments with the materials.

Residues may be found on the surface or absorbed by plant material. They may include the toxicant, closely related products present in the technical toxicant, degradation products, and metabolites. The quantitative estimation of insecticide residues is important as a supplement to entomological performance records and as an estimate of the magnitude of food contamination.

It is pointed out that residue data secured as a result of belated decisions from an experiment designed to accrue biological data may lead to misleading or erroneous conclusions. Factors to be considered in sampling are the type of commodity, history of previous treatment to plants or soil in experimental area, biological variations, formulations of pesticides, uniformity of application, and environmental factors, including geographical distribution.

Samples must be adequate in size and sufficiently well randomized to be representative of the commodity. As a general rule, each field plot should be replicated at least three times and there should be at least three sample replicates per plot. In the study of degradation and persistence of pesticide residues, timing of sample collection may be critical.

Prudent management of an analytical program would insure analysis of samples as quickly as possible. This will require close cooperation between the entomologist, conducting field experiments, and the analyst.

Sample processing means the transfer of the insecticide by mechanical or physical means from the plant to a suitable solvent for analysis. This includes the processes of subdividing and sampling of the original sample, treatment of the subsample with the proper solvents to effectively dissolve the insecticide from the substrate, and storage of the sample solution.

Clean-up processes for the removal of extractives which would interfere in residue analysis are discussed. These processes include chromatography, oxidation, reduction, saponification, acid washes, combustion, selective solvent separation, steam distillation, and others.

Analytical processes applied in residue analyses include chemical analysis; bioassays using living organisms, including insects and fish; enzymatic methods; physical examination, including ultraviolet and infrared spectra, polarographic assay, and colorimetry. The use of "check," "control," and "fortified" samples to evaluate analytical techniques is discussed.

Data obtained in the study of insecticide residues and means of interpreting these data are discussed. It is pointed out that the skill and experience of the analyst are often of vital importance in the interpretation of these data.

A system of analytical bookkeeping and its importance in studies of insecticide residues is discussed.

Safety programs which should be observed in working with insecticides and volatile solvents so as to reduce health hazards to a minimum are discussed. Suggestions for adequate ventilation of laboratory space and rooms are made.

In the second section of this book, one chapter is devoted to problems encountered in analysis of pesticide formulations. The authors do not attempt to suggest solutions to all of these problems.

The third section of this book could well serve as a text for study of insecticide residues. This section includes specific directions for sampling (so far as they are available) many agricultural crops; a discussion of methods for measurement of samples of crops and plant parts; and an outline of methods of "clean up" employed to isolate the residue prior to final analysis. In addition, the final chapter contains detailed methods for analysis of many pesticide residues.

This book represents a tremendous effort in assembling information into a well organized text. It is an excellent introduction to the spray residue literature and deserves a place in the library of everyone interested in pesticide chemicals.

J. E. FAHEY

DDT—DAS INSEKTIZID DICHLOR DIPHENYLTRICHLORÄTHAN UND SEINE BEDEUTUNG, by Paul Müller. Verlag Birkhauser, Basel. Vol. I, 299 pp. illus. 37.5 Swiss francs.

This is the first of a projected 3-volume monograph on DDT, edited by Nobel prize-winner Paul Müller. It is appropriate that the discoverer of the insecticidal properties of DDT should edit this work as well as contribute a chapter on the physics and chemistry of DDT. Other chapters in Vol. I are the Mode of Action of DDT (19 pages) by V. B. Wigglesworth of Cambridge; the Application of DDT in the Protection of Grain (29 pages) by E. Bernfus of Vienna; the Application of DDT Insecticides for the Protection of Textiles (49 pages) by O. Wälchli of St. Gallen; and the Application of DDT in Forestry (100 pages) by V. Butovitsch of Stockholm.

Volume II of this series, scheduled to appear in 1956, will contain chapters on the Pharmacology and Toxicology of DDT by W. J. Hayes of the U. S. Public Health Service; the Use of DDT Insecticides in Human Medicine by S. W. Simmons, also of the U. S. Public Health Service; the Use of DDT in Veterinary Medicine by E. F. Knipling of the U. S. Department of Agriculture.

Volume III, to appear in 1957, will contain information on the application of DDT in plant protection (author not stated) and a general summary by Müller.

Volume I describes the discovery of the insecticidal value of DDT and its early development in an authoritative manner; likewise the physical and chemical properties, such as solubility, are exhaustively discussed. Analytical methods receive adequate attention. A table is given listing many analogs of DDT with structural formulas and references to the original literature.

Some of the disappointing features of this book are the very brief discussion of insect resistance to DDT; the failure to describe adequately the process of large scale manufacture of DDT; the omission of statistics on the production, consumption and world trade in DDT insecticides, amounting to many millions of pounds annually; and the failure to point out that the value of DDT for practical insect control depends largely on its proper formulation and to describe the preparation of suitable solutions, emulsions, wettable powders and dusts.

The American reader of this book will be startled to learn that Geigy 33 (containing 10 per cent of DDT) is officially sanctioned in Australia for dusting wheat infested with grain weevils and other insect pests at the rate of 1:1000. This would deposit DDT on the wheat to the extent of 100 p.p.m. Subsequent dilution with untreated grain reduces but does not eliminate this DDT residue. It is hoped that DDT residues on fruits and vegetables and in meat and milk and their hazard to the consumer will receive adequate attention in the forthcoming volumes.

No reference is made to the recent work of American entomologists in mothproofing wool with DDT, probably because Walchli's chapter was completed in December, 1952.

Volume I of this work on DDT will prove valuable to the insect physiologist and to the insecticide chemist working on the mode of action of insecticides because of its full theoretical treatment of toxicity and chemical structure, accompanied by a wealth of references.

R. C. ROARK

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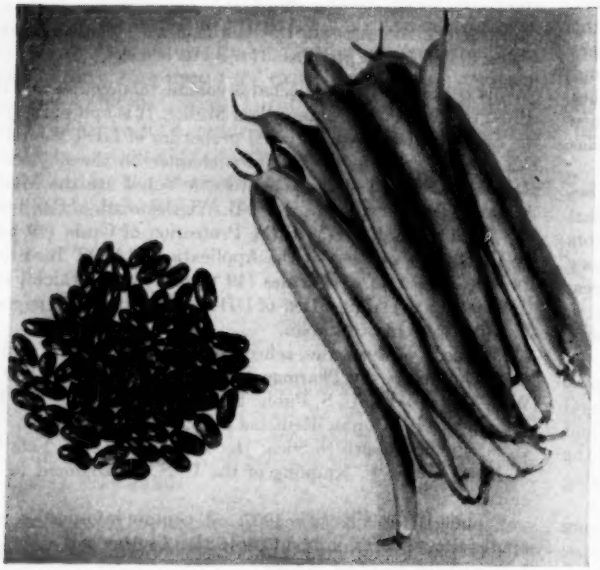

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